

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
23 October 2003 (23.10.2003)

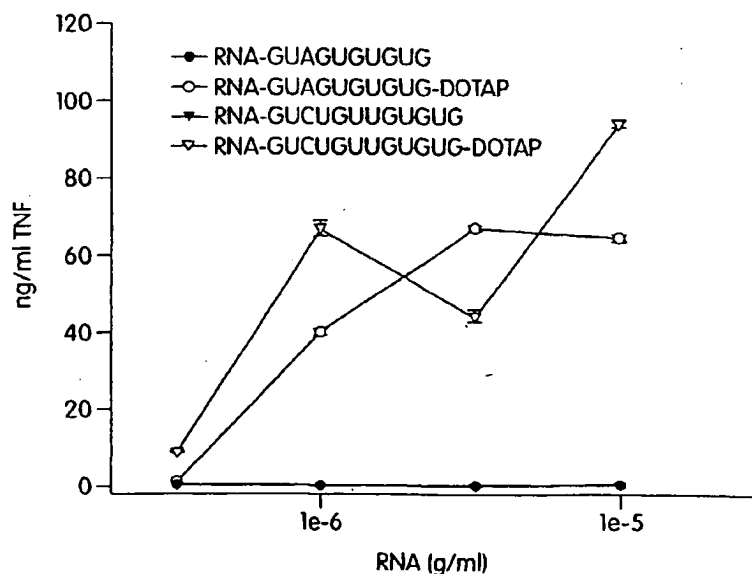
PCT

(10) International Publication Number  
WO 03/086280 A2

- (51) International Patent Classification<sup>7</sup>: **A61K**
- (21) International Application Number: PCT/US03/10406
- (22) International Filing Date: 4 April 2003 (04.04.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
60/370,515 4 April 2002 (04.04.2002) US  
60/421,966 29 October 2002 (29.10.2002) US
- (71) Applicant (for all designated States except US): **COLEY PHARMACEUTICAL GMBH [DE/DE]**; Elisabeth-Selbert-Strasse 9, 40764 Langenfeld (DE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **LIPFORD, Grayson [US/DE]**; Benrode Strasse 86, 40597 Duesseldorf (DE). **BAUER, Stefan [DE/DE]**; Waisenhausstrasse 13, 80637 Munchen (DE).
- (74) Agent: **STEELE, Alan, W.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: IMMUNOSTIMULATORY G,U-CONTAINING OLIGORIBONUCLEOTIDES



(57) Abstract: Compositions and methods relating to immunostimulatory RNA oligomers are provided. The immunostimulatory RNA molecules are believed to represent natural ligands of one or more Toll-like receptors, including Toll-like receptor 7 (TLR7) and Toll-like receptor 8 (TLR8). The compositions and methods are useful for stimulating immune activation. Methods useful for screening candidate immunostimulatory compounds are also provided.

WO 03/086280 A2



---

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## **IMMUNOSTIMULATORY G,U-CONTAINING OLIGORIBONUCLEOTIDES**

### **Field of the Invention**

The present invention relates generally to the field of immunology and immune stimulation. More particularly, the present invention relates to immunostimulatory ribonucleic acids, homologs of said immunostimulatory ribonucleic acids, and methods of use of said immunostimulatory ribonucleic acids and homologs. Compositions and methods of the invention are believed to be useful for inducing signaling through Toll-like receptor 7 (TLR7) and Toll-like receptor 8 (TLR8).

### **Background of the Invention**

The immune response is conceptually divided into innate immunity and adaptive immunity. Innate immunity is believed to involve recognition of pathogen-associated molecular patterns (PAMPs) shared in common by certain classes of molecules expressed by infectious microorganisms or foreign macromolecules. PAMPs are believed to be recognized by pattern recognition receptors (PRRs) on certain immune cells.

Toll-like receptors (TLRs) are a family of highly conserved polypeptides that play a critical role in innate immunity in mammals. Currently ten family members, designated TLR1 - TLR10, have been identified. The cytoplasmic domains of the various TLRs are characterized by a Toll-interleukin 1 (IL-1) receptor (TIR) domain. Medzhitov R et al. (1998) *Mol Cell* 2:253-8. Recognition of microbial invasion by TLRs triggers activation of a signaling cascade that is evolutionarily conserved in *Drosophila* and mammals. The TIR domain-containing adapter protein MyD88 has been reported to associate with TLRs and to recruit IL-1 receptor-associated kinase (IRAK) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) to the TLRs. The MyD88-dependent signaling pathway is believed to lead to activation of NF- $\kappa$ B transcription factors and c-Jun NH<sub>2</sub> terminal kinase (Jnk) mitogen-activated protein kinases (MAPKs), critical steps in immune activation and production of inflammatory cytokines. For a review, see Aderem A et al. (2000) *Nature* 406:782-87.

While a number of specific TLR ligands have been reported, ligands for some TLRs remain to be identified. Ligands for TLR2 include peptidoglycan and lipopeptides. Yoshimura A et al. (1999) *J Immunol* 163:1-5; Yoshimura A et al. (1999) *J Immunol* 163:1-5;

- 2 -

Aliprantis AO et al. (1999) *Science* 285:736-9. Viral-derived double-stranded RNA (dsRNA) and poly I:C, a synthetic analog of dsRNA, have been reported to be ligands of TLR3.

Alexopoulou L et al. (2001) *Nature* 413:732-8. Lipopolysaccharide (LPS) is a ligand for TLR4. Poltorak A et al. (1998) *Science* 282:2085-8; Hoshino K et al. (1999) *J Immunol*

5 162:3749-52. Bacterial flagellin is a ligand for TLR5. Hayashi F et al. (2001) *Nature* 410:1099-1103. Peptidoglycan has been reported to be a ligand not only for TLR2 but also for TLR6. Ozinsky A et al. (2000) *Proc Natl Acad Sci USA* 97:13766-71; Takeuchi O et al. (2001) *Int Immunol* 13:933-40. Bacterial DNA (CpG DNA) has been reported to be a TLR9 ligand. Hemmi H et al. (2000) *Nature* 408:740-5; Bauer S et al. (2001) *Proc Natl Acad Sci*  
10 *USA* 98, 9237-42. The TLR ligands listed above all include natural ligands, i.e., TLR ligands found in nature as molecules expressed by infectious microorganisms.

The natural ligands for TLR1, TLR7, TLR8 and TLR10 are not known, although recently certain low molecular weight synthetic compounds, the imidazoquinolones imiquimod (R-837) and resiquimod (R-848), were reported to be ligands of TLR7. Hemmi H  
15 et al. (2002) *Nat Immunol* 3:196-200.

### Summary of the Invention

The present invention is based in part on the novel discovery by the inventors of certain immunostimulatory RNA and RNA-like (hereinafter, simply "RNA") molecules. The  
20 immunostimulatory RNA molecules of the invention are believed by the inventors to require a base sequence that includes at least one guanine (G) and at least one uracil (U), wherein optionally the at least one G can be a variant or homolog of G and/or the at least one U can independently be a variant or homolog of U. Surprisingly, the immunostimulatory RNA molecules of the invention can be either single-stranded or at least partially double-stranded.  
25 Also surprisingly, the immunostimulatory RNA molecules of the invention do not require a CpG motif in order to exert their immunostimulatory effect. Without meaning to be bound by any particular theory or mechanism, it is the belief of the inventors that the immunostimulatory RNA molecules of the invention signal through an MyD88-dependent pathway, probably through a TLR. Also without meaning to be bound by any particular  
30 theory or mechanism, it is the belief of the inventors that the immunostimulatory RNA molecules of the invention interact with and signal through TLR8, TLR7, or some other TLR yet to be identified.

- 3 -

The immunostimulatory RNA molecules of the invention are also believed by the inventors to be representative of a class of RNA molecules, found in nature, which can induce an immune response. Without meaning to be bound by any particular theory or mechanism, it is the belief of the inventors that the corresponding class of RNA molecules found in nature is believed to be present in ribosomal RNA (rRNA), transfer RNA (tRNA), messenger RNA (mRNA), and viral RNA (vRNA). It is to be noted in this regard that the immunostimulatory RNA molecules of the present invention can be as small as 5-40 nucleotides long. Such short RNA molecules fall outside the range of full length messenger RNAs described to be useful in transfecting dendritic cells in order to induce an immune response to cancer antigens. See, e.g., Boczkowski D et al. (1996) *J Exp Med* 184:465-72; Mitchell DA et al. (2000) *Curr Opin Mol Ther* 2:176-81.

It has also been discovered according to the present invention that the immunostimulatory RNA molecules of the invention can be advantageously combined with certain agents which promote stabilization of the RNA, local clustering of the RNA molecules, and/or trafficking of the RNA molecules into the endosomal compartment of cells. In particular, it has been discovered according to the present invention that certain lipids and/or liposomes are useful in this regard. For example, certain cationic lipids, including in particular N-[1-(2, 3 dioleoyloxy)-propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP), appear to be especially advantageous when combined with the immunostimulatory RNA molecules of the invention. As another example, covalent conjugation of a cholesteryl moiety to the RNA, for example to the 3' end of the RNA, promotes the immunostimulatory effect of the RNA, even in the absence of cationic lipid.

The invention provides compositions of matter and methods related to the immunostimulatory RNA molecules of the invention. The compositions and methods are useful, inter alia, for activating immune cells in vivo, in vitro, and ex vivo; treating infection; treating cancer; preparing a pharmaceutical composition; identifying a target receptor for the immunostimulatory RNA; and screening for and characterizing additional immunostimulatory compounds. Furthermore, the compositions of matter and methods related to the immunostimulatory RNA molecules of the instant invention can advantageously be combined with other immunostimulatory compositions of matter and methods related to such other immunostimulatory compositions of matter.

- 4 -

In one aspect the invention provides an immunostimulatory composition. The immunostimulatory composition according to this aspect of the invention includes an isolated RNA oligomer 5-40 nucleotides long having a base sequence having at least one guanine (G) and at least one uracil (U), and optionally a cationic lipid. The RNA oligomer can be of  
5 natural or non-natural origin. An RNA oligomer of natural origin can in one embodiment be derived from prokaryotic RNA and in another embodiment can be derived from eukaryotic RNA. In addition, the RNA oligomer of natural origin can include a portion of a ribosomal RNA. An RNA oligomer of non-natural origin can include an RNA molecule synthesized outside of a cell, e.g., using chemical techniques known by those of skill in the art. In one  
10 embodiment an RNA oligomer can include a derivative of an RNA oligomer of natural origin.

In one embodiment the isolated RNA oligomer is a G,U-rich RNA as defined below.

In one embodiment the G,U-containing immunostimulatory RNA is an isolated RNA molecule at least 5 nucleotides long which includes a base sequence as provided by  
15 5'-RURGY-3', wherein R represents purine, U represents uracil, G represents guanine, and Y represents pyrimidine. In one embodiment the G,U-containing immunostimulatory RNA is an isolated RNA molecule at least 5 nucleotides long which includes a base sequence as provided by 5'-GUAGU-3', wherein A represents adenine. In one embodiment the G,U-containing immunostimulatory RNA is an isolated RNA molecule which includes a base  
20 sequence as provided by 5'-GUAGUGU-3'.

In one embodiment the G,U-containing immunostimulatory RNA is an isolated RNA molecule at least 5 nucleotides long which includes a base sequence as provided by 5'-GUUGB-3', wherein B represents U, G, or C.

In one embodiment the G,U-containing immunostimulatory RNA is an isolated RNA  
25 molecule at least 5 nucleotides long which includes a base sequence as provided by 5'-GUGUG-3'.

In other embodiments the isolated RNA molecule can contain multiples of any of the foregoing sequences, combinations of any of the foregoing sequences, or combinations of any of the foregoing sequences including multiples of any of the foregoing sequences. The  
30 multiples and combinations can be linked directly or they can be linked indirectly, i.e., through an intervening nucleoside or sequence. In one embodiment the intervening linking nucleoside is G; in one embodiment the intervening linking nucleoside is U.

- 5 -

In one embodiment the base sequence includes 5'-GUGUUUAC-3'. In one embodiment the base sequence is 5'-GUGUUUAC-3'.

In another embodiment the the base sequence includes 5'-GUAGGCAC-3'. In one embodiment the the base sequence is 5'-GUAGGCAC-3'.

5 In yet another embodiment the base sequence includes 5'-CUAGGCAC-3'. In one embodiment the base sequence is 5'-CUAGGCAC-3'.

In still another embodiment the base sequence includes 5'-CUCGGCAC-3'. In one embodiment the base sequence is 5'-CUCGGCAC-3'.

10 In one embodiment the oligomer is 5-12 nucleotides long. In one embodiment the oligomer is 8-12 nucleotides long.

Also according to this aspect of the invention, in one embodiment the base sequence is free of CpG dinucleotide. Thus in this embodiment the immunostimulatory RNA is not a CpG nucleic acid.

In certain embodiments according to this aspect of the invention, the base sequence of  
15 the RNA oligomer is at least partially self-complementary. In one embodiment the extent of self-complementarity is at least 50 percent. The extent of self-complementarity can extend to and include 100 percent. Thus for example the base sequence of the at least partially self-complementary RNA oligomer in various embodiments can be at least 50 percent, at least 60 percent, at least 70 percent, at least 80 percent, at least 90 percent, or 100 percent self-  
20 complementary. Complementary base pairs include guanine-cytosine (G-C), adenine-uracil (A-U), adenine-thymine (A-T), and guanine-uracil (G-U). G-U "wobble" basepairing, which is fairly common in ribosomal RNA and in RNA retroviruses, is somewhat weaker than traditional Watson-Crick basepairing between G-C, A-T, or A-U. A partially self-complementary sequence can include one or more portions of self-complementary sequence.  
25 In an embodiment which involves a partially self-complementary sequence, the RNA oligomer can include a self-complementary portion positioned at and encompassing each end of the oligomer.

In one embodiment according to this aspect of the invention, the oligomer is a plurality of oligomers, i.e., a plurality of RNA oligomers each 6-40 nucleotides long having a  
30 base sequence comprising at least one guanine (G) and at least one uracil (U). The plurality of oligomers can, but need not, include sequences which are at least partially complementary to one another. In one embodiment the plurality of oligomers includes an oligomer having a

- 6 -

first base sequence and an oligomer having a second base sequence, wherein the first base sequence and the second base sequence are at least 50 percent complementary. Thus for example the at least partially complementary base sequences in various embodiments can be at least 50 percent, at least 60 percent, at least 70 percent, at least 80 percent, at least 90 percent, or 100 percent complementary. As described above, complementary base pairs include guanine-cytosine (G-C), adenine-uracil (A-U), adenine-thymine (A-T), and guanine-uracil (G-U). Partially complementary sequences can include one or more portions of complementary sequence. In an embodiment which involves partially complementary sequences, the RNA oligomers can include a complementary portion positioned at and encompassing at least one end of the oligomers.

In one embodiment the oligomer is a plurality of oligomers which includes an oligomer having a base sequence including 5'-GUGUUUAC-3' and an oligomer having a base sequence including 5'-GUAGGCAC-3'. In one embodiment the oligomer is a plurality of oligomers which includes an oligomer having a base sequence 5'-GUGUUUAC-3' and an oligomer having a base sequence 5'-GUAGGCAC-3'.

Further according to this aspect of the invention, in various embodiments the oligomer includes a non-natural backbone linkage, a modified base, a modified sugar, or any combination of the foregoing. The non-natural backbone linkage can be a stabilized linkage, i.e., a linkage which is relatively resistant against RNase or nuclease degradation, compared with phosphodiester linkage. In one embodiment the non-natural backbone linkage is a phosphorothioate linkage. The oligomer can include one non-natural backbone linkage or a plurality of non-natural backbone linkages, each selected independently of the rest. The modified base can be a modified G, U, A, or C, including the at least one G and the at least one U of the base sequence according to this aspect of the invention. In some embodiments the modified base can be selected from 7-deazaguanosine, 8-azaguanosine, 5-methyluracil, and pseudouracil. The oligomer can include one modified base or a plurality of modified bases, each selected independently of the rest. The modified sugar can be a methylated sugar, arabinose. The oligomer can include one modified sugar or a plurality of modified sugars, each selected independently of the rest.

In one embodiment the cationic lipid is N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP). DOTAP is believed to transport RNA oligomer into cells and specifically traffic to the endosomal compartment, where it can

- 7 -

release the RNA oligomer in a pH-dependent fashion. Once in the endosomal compartment, the RNA can interact with certain intracellular Toll-like receptor molecules (TLRs), triggering TLR-mediated signal transduction pathways involved in generating an immune response. Other agents with similar properties including trafficking to the endosomal  
5 compartment can be used in place of or in addition to DOTAP.

In one embodiment the immunostimulatory composition further includes an antigen. In one embodiment the antigen is an allergen. In one embodiment the antigen is a cancer antigen. In one embodiment the antigen is a microbial antigen.

Also according to this aspect of the invention, in another embodiment the invention is  
10 a pharmaceutical composition. The pharmaceutical composition includes an immunostimulatory composition of the invention and a pharmaceutically acceptable carrier. Methods for preparing the pharmaceutical composition are also provided. Such methods entail placing an immunostimulatory composition of the invention in contact with a pharmaceutically acceptable carrier. The pharmaceutical composition can be formulated in a  
15 unit dosage for convenience.

In another aspect the invention provides a method of activating an immune cell. The method involves contacting an immune cell with an immunostimulatory composition of the invention, described above, in an effective amount to induce activation of the immune cell. In one embodiment the activation of the immune cell involves secretion of a cytokine by the  
20 immune cell. The cytokine in one embodiment is selected from the group consisting of interleukin 6 (IL-6), interleukin 12 (IL-12), an interferon (IFN), and tumor necrosis factor (TNF). In one embodiment the activation of the immune cell includes secretion of a chemokine. In one embodiment the secreted chemokine is interferon-gamma-induced protein 10 (IP-10). In one embodiment the activation of the immune cell includes expression of a  
25 costimulatory/accessory molecule by the immune cell. In one embodiment the costimulatory/accessory molecule is selected from the group consisting of intercellular adhesion molecules (ICAMs, e.g., CD54), leukocyte function-associated antigens (LFAs, e.g., CD58), B7s (CD80, CD86), and CD40.

Also according to this aspect of the invention, in one embodiment the activation of the  
30 immune cell involves activation of a MyD88-dependent signal transduction pathway. MyD88 is believed to be an adapter molecule that interacts with the Toll/interleukin-1 receptor (TIR) domain of various Toll-like receptor (TLR) molecules and participates in

- 8 -

signal transduction pathways that ultimately result in activation of nuclear factor kappa B (NF- $\kappa$ B). Thus in one embodiment the MyD88-dependent signal transduction pathway is associated with a TLR. More particularly, in one embodiment the TLR is TLR8. In another embodiment the TLR is TLR7.

5 Also according to this aspect of the invention in one embodiment the immune cell is a human immune cell. The immune cell in one embodiment is a myeloid dendritic cell.

In one embodiment of this aspect of the invention the contacting occurs in vitro. In another embodiment the contacting occurs in vivo.

The invention in another aspect provides a method of inducing an immune response in  
10 a subject. The method according to this aspect of the invention involves administering to a subject an immunostimulatory composition of the invention in an effective amount to induce an immune response in the subject. It is to be noted that the method according to this aspect of the invention does not involve administration of an antigen to the subject. In one embodiment the subject is a human. In one embodiment the subject has or is at risk of having  
15 a cancer. In one embodiment the subject has or is at risk of having an infection with an agent selected from the group consisting of viruses, bacteria, fungi, and parasites. In a particular embodiment the subject has or is at risk of having a viral infection. It is also to be noted that the method according to this aspect of the invention can be used to treat a subject with a suppressed capacity to mount an effective or desirable immune response. For example the  
20 subject can have a suppressed immune system due to an infection, a cancer, an acute or chronic disease such as kidney or liver insufficiency, surgery, and an exposure to an immunosuppressive agent such as chemotherapy, radiation, certain drugs, or the like. In one embodiment the subject has or is at risk of having an allergy or asthma. Such a subject can be exposed to or at risk of exposure to an allergen that is associated with an allergic response  
25 or asthma in the subject.

In yet another aspect the invention provides a method of inducing an immune response in a subject. The method according to this aspect of the invention involves administering an antigen to a subject, and administering to the subject an immunostimulatory composition of the invention in an effective amount to induce an immune response to the  
30 antigen. It is to be noted that the antigen can be administered before, after, or concurrently with the immunostimulatory composition of the invention. In addition, both the antigen and the immunostimulatory compound can be administered to the subject more than once.

- 9 -

In one embodiment according to this aspect of the invention the antigen is an allergen.  
In one embodiment according to this aspect of the invention the antigen is a cancer antigen.  
The cancer antigen in one embodiment can be a cancer antigen isolated from the subject. In  
another embodiment the antigen is a microbial antigen. The microbial antigen can be an  
5 antigen of a virus, a bacterium, a fungus, or a parasite.

The invention further provides, in yet another aspect, a method of inducing an  
immune response in a subject. The method according to this aspect of the invention involves  
isolating dendritic cells of a subject, contacting the dendritic cells ex vivo with an  
immunostimulatory composition of the invention, contacting the dendritic cells ex vivo with  
10 an antigen, and administering the contacted dendritic cells to the subject.

In one embodiment according to this aspect of the invention the antigen is an allergen.  
In one embodiment according to this aspect of the invention the antigen is a cancer antigen.  
The cancer antigen in one embodiment can be a cancer antigen isolated from the subject. In  
another embodiment the antigen is a microbial antigen. The microbial antigen can be an  
15 antigen of a virus, a bacterium, a fungus, or a parasite.

An immune response arising from stimulation of one TLR can be modified, enhanced  
or amplified by stimulation of another TLR, and the combined immunostimulatory effect  
may be synergistic. For example, TLR9 is reported to respond to bacterial DNA and, more  
generally, CpG DNA. An immune response arising from TLR9 contacting its natural ligand  
20 (or any TLR9 ligand) may be modified, enhanced or amplified by also selectively contacting  
TLR7 with a TLR7 ligand, or by also selectively contacting TLR8 with a TLR8 ligand, or  
both. Likewise, an immune response arising from TLR7 contacting a TLR7 ligand may be  
modified, enhanced or amplified by also selectively contacting TLR8 with a TLR8 ligand, or  
by also selectively contacting TLR9 with CpG DNA (or any suitable TLR9 ligand), or both.  
25 As yet another example, an immune response arising from TLR8 contacting a TLR8 ligand  
may be modified, enhanced or amplified by also selectively contacting TLR7 with a TLR7  
ligand, or by also selectively contacting TLR9 with CpG DNA (or any suitable TLR9 ligand),  
or both.

The present invention is based in part on the novel discovery by the inventors of what  
30 are believed to be natural ligands for TLR7 and TLR8. While naturally occurring ligands  
derived from microbes have been described for certain TLRs, natural ligands for TLR7 and  
TLR8 have not previously been described. Certain synthetic small molecules,

- 10 -

imidazoquinoline compounds, have been described as ligands for TLR7, but such compounds are to be distinguished from the natural ligands of the present invention. Hemmi H et al. (2002) *Nat Immunol* 3:196-200.

Isolated natural ligands of TLR7 and TLR8 are useful as compositions that can induce, enhance, and complement an immune response. The natural ligands of TLR7 and TLR8 are useful for preparation of novel compositions that can induce, enhance, and complement an immune response. In addition, the natural ligands of TLR7 and TLR8 are useful for selectively inducing TLR7- and TLR8-mediated signaling and for selectively inducing TLR7- and TLR8-mediated immune responses. Furthermore, the natural ligands of TLR7 and TLR8 are useful in designing and performing screening assays for identification and selection of immunostimulatory compounds.

The present invention is also based in part on the novel discovery according to the invention that human neutrophils strongly express TLR8. This observation is important because neutrophils are very often the first cells to engage infectious pathogens and thus to initiate responses. It is believed that activated neutrophils secrete chemokines and cytokines, which in turn are instrumental in recruiting dendritic cells. TLR9-expressing dendritic cells drawn to the site of the activated neutrophils there become activated, thereby amplifying the immune response.

The present invention is also based in part on the appreciation of the differential expression of various TLRs, including TLR7, TLR8, and TLR9, on various cells of the immune system. This segregation may be of particular significance in humans with respect to TLR7, TLR8, and TLR9. The immune response arising from stimulation of any one of these TLRs may be enhanced or amplified by stimulation of another TLR, and the combined immunostimulatory effect may be synergistic. For example, TLR9 is reported to respond to bacterial DNA and, more generally, CpG DNA. An immune response arising from TLR9 contacting its natural ligand (or any TLR9 ligand) may be enhanced or amplified by also selectively contacting TLR7 with its natural ligand (or any suitable TLR7 ligand), or by also selectively contacting TLR8 with its natural ligand (or any suitable TLR8 ligand), or both. Likewise, an immune response arising from TLR7 contacting its natural ligand (or any TLR7 ligand) may be enhanced or amplified by also selectively contacting TLR8 with its natural ligand (or any suitable TLR8 ligand), or by also selectively contacting TLR9 with CpG DNA (or any suitable TLR9 ligand), or both. As yet another example, an immune response arising

- 11 -

from TLR8 contacting its natural ligand (or any TLR8 ligand) may be enhanced or amplified by also selectively contacting TLR7 with its natural ligand (or any suitable TLR7 ligand), or by also selectively contacting TLR9 with CpG DNA (or any suitable TLR9 ligand), or both.

In a further aspect the invention provides a composition including an effective amount  
5 of a ligand for TLR8 to induce TLR8 signaling and an effective amount of a ligand for a second TLR selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR9 and TLR10 to induce signaling by the second TLR. In one embodiment the second TLR is TLR3. In one embodiment the second TLR is TLR7. In one embodiment the second TLR is TLR9. In one embodiment the ligand for TLR8 and the ligand for the  
10 second TLR are linked. In yet another embodiment the composition further includes a pharmaceutically acceptable carrier.

In another aspect the invention provides a composition including an effective amount of a ligand for TLR7 to induce TLR7 signaling and an effective amount of a ligand for a second TLR selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5,  
15 TLR6, TLR8, TLR9, and TLR10 to induce signaling by the second TLR. In one embodiment the second TLR is TLR3. In one embodiment the second TLR is TLR8. In one embodiment the second TLR is TLR9. In one embodiment the ligand for TLR7 and the ligand for the second TLR are linked. In yet another embodiment the composition further includes a pharmaceutically acceptable carrier.

In a further aspect the invention provides a composition including a DNA:RNA  
20 conjugate, wherein DNA of the conjugate includes an immunostimulatory motif effective for stimulating TLR9 signaling and wherein RNA of the conjugate includes RNA effective for stimulating signaling by TLR3, TLR7, TLR8, or any combination thereof. In one embodiment the immunostimulatory motif effective for stimulating TLR9 signaling is a CpG motif. In another embodiment the immunostimulatory motif effective for stimulating TLR9  
25 signaling is poly-dT. In yet another embodiment the immunostimulatory motif effective for stimulating TLR9 signaling is poly-dG. In one embodiment the conjugate includes a chimeric DNA:RNA backbone. In one embodiment the chimeric backbone includes a cleavage site between the DNA and the RNA. In one embodiment the conjugate includes a  
30 double-stranded DNA:RNA heteroduplex. In yet another embodiment the composition further includes a pharmaceutically acceptable carrier.

- 12 -

In another aspect the invention provides a method for stimulating TLR8 signaling. The method involves contacting TLR8 with an isolated RNA in an effective amount to stimulate TLR8 signaling. In one embodiment the RNA is double-stranded RNA. In one embodiment the RNA is ribosomal RNA. In one embodiment the RNA is transfer RNA. In one embodiment the RNA is messenger RNA. In one embodiment the RNA is viral RNA. In one embodiment the RNA is G,U-rich RNA. In one embodiment the RNA consists essentially of G and U.

In yet another aspect the invention provides a method for stimulating TLR8 signaling. The method according to this aspect involves contacting TLR8 with a mixture of nucleosides consisting essentially of G and U in a ratio between 1G:50U and 10G:1U, in an amount effective to stimulate TLR8 signaling. In one embodiment the nucleosides are ribonucleosides. In one embodiment the nucleosides comprise a mixture of ribonucleosides and deoxyribonucleosides. In one embodiment the G is a guanosine derivative selected from the group consisting of: 8-bromoguanosine, 8-oxoguanosine, 8-mercaptoguanosine, 7-allyl-8-oxoguanosine, guanosine ribonucleoside vanadyl complex, inosine, and nebularine.

A further aspect of the invention provides a method for stimulating TLR8 signaling. The method according to this aspect involves contacting TLR8 with a mixture of ribonucleoside vanadyl complexes. In one embodiment the mixture comprises guanosine ribonucleoside vanadyl complexes.

In another aspect the invention provides a method for stimulating TLR8 signaling. The method according to this aspect involves contacting TLR8 with an isolated G,U-rich oligonucleotide comprising a sequence selected from the group consisting of: UUGUGG, UGGUUG, GUGUGU, and GGGUUU, in an amount effective to stimulate TLR8 signaling. In one embodiment the oligonucleotide is an oligoribonucleotide. In one embodiment the oligonucleotide is 7-50 bases long. In one embodiment the oligonucleotide is 12-24 bases long. In one embodiment the oligonucleotide has a sequence 5'-GUUGUGGUUGUGGUUGUG-3' (SEQ ID NO:1).

The invention provides in another aspect a method for stimulating TLR8 signaling. The method according to this aspect involves contacting TLR8 with an at least partially double-stranded nucleic acid molecule comprising at least one G-U base pair, in an amount effective to stimulate TLR8 signaling.

- 13 -

In yet another aspect the invention provides a method for supplementing a TLR8-mediated immune response. The method involves contacting TLR8 with an effective amount of a TLR8 ligand to induce a TLR8-mediated immune response, and contacting a TLR other than TLR8 with an effective amount of a ligand for the TLR other than TLR8 to induce an immune response mediated by the TLR other than TLR8.

In a further aspect the invention provides a method for supplementing a TLR8-mediated immune response in a subject. The method according to this aspect involves administering to a subject in need of an immune response an effective amount of a TLR8 ligand to induce a TLR8-mediated immune response, and administering to the subject an effective amount of a ligand for a TLR other than TLR8 to induce an immune response mediated by the TLR other than TLR8. In one embodiment the TLR other than TLR8 is TLR9. In one embodiment the ligand for TLR9 is a CpG nucleic acid. In one embodiment the CpG nucleic acid has a stabilized backbone. In one embodiment the ligand for TLR8 and the ligand for TLR9 are a conjugate. In one embodiment the conjugate comprises a double-stranded DNA:RNA heteroduplex. In one embodiment the conjugate comprises a chimeric DNA:RNA backbone. In one embodiment the chimeric backbone comprises a cleavage site between the DNA and the RNA.

The invention in a further aspect provides a method for stimulating TLR7 signaling. The method according to this aspect involves contacting TLR7 with an isolated guanosine ribonucleoside in an effective amount to stimulate TLR7 signaling. In one embodiment the guanosine ribonucleoside is a guanosine ribonucleoside derivative selected from the group consisting of: 8-bromoguanosine, 8-oxoguanosine, 8-mercaptopguanosine, 7-allyl-8-oxoguanosine, guanosine ribonucleoside vanadyl complex, inosine, and nebularine. In one embodiment the guanosine ribonucleoside derivative is 8-oxoguanosine. In one embodiment the guanosine nucleoside is a ribonucleoside. In one embodiment the guanosine nucleoside comprises a mixture of ribonucleosides and deoxyribonucleosides.

In another aspect the invention further provides a method for stimulating TLR7 signaling. The method according to this aspect involves contacting TLR7 with an isolated nucleic acid comprising a terminal oxidized or halogenized guanosine in an effective amount to stimulate TLR7 signaling. In one embodiment the oxidized or halogenized guanosine is 8-oxoguanosine.

- 14 -

In another aspect the invention provides a method for stimulating TLR7 signaling. The method according to this aspect involves contacting TLR7 with an isolated RNA in an effective amount to stimulate TLR7 signaling. In one embodiment the RNA is double-stranded RNA. In one embodiment the RNA is ribosomal RNA. In one embodiment the RNA is transfer RNA. In one embodiment the RNA is messenger RNA. In one embodiment the RNA is viral RNA. In one embodiment the RNA is G-rich RNA. In one embodiment the RNA is part of a DNA:RNA heteroduplex. In one embodiment the RNA consists essentially of guanosine ribonucleoside.

The invention in yet another aspect provides a method for stimulating TLR7 signaling. The method according to this aspect involves contacting TLR7 with a mixture of nucleosides consisting essentially of G and U in a ratio between 1G:50U and 10G:1U, in an amount effective to stimulate TLR7 signaling.

Provided in yet another aspect of the invention is a method for stimulating TLR7 signaling. The method according to this aspect involves contacting TLR7 with a mixture of ribonucleoside vanadyl complexes. In one embodiment the mixture comprises guanosine ribonucleoside vanadyl complexes.

In a further aspect the invention provides a method for supplementing a TLR7-mediated immune response. The method according to this aspect involves contacting TLR7 with an effective amount of a TLR7 ligand to induce a TLR7-mediated immune response, and contacting a TLR other than TLR7 with an effective amount of a ligand for the TLR other than TLR7 to induce an immune response mediated by the TLR other than TLR7.

In yet another aspect the invention provides a method for supplementing a TLR7-mediated immune response in a subject. The method involves administering to a subject in need of an immune response an effective amount of a TLR7 ligand to induce a TLR7-mediated immune response, and administering to the subject an effective amount of a ligand for a TLR other than TLR7 to induce an immune response mediated by the TLR other than TLR7. In one embodiment the TLR other than TLR7 is TLR9. In one embodiment the ligand for TLR9 is a CpG nucleic acid. In one embodiment the CpG nucleic acid has a stabilized backbone. In one embodiment the ligand for TLR7 and the ligand for TLR9 are a conjugate. In one embodiment the conjugate comprises a double-stranded DNA:RNA heteroduplex. In one embodiment the conjugate comprises a chimeric DNA:RNA backbone.

- 15 -

In one embodiment the chimeric backbone comprises a cleavage site between the DNA and the RNA.

The invention in another aspect provides a method for screening candidate immunostimulatory compounds. The method according to this aspect involves measuring a  
5 TLR8-mediated reference signal in response to an RNA reference, measuring a TLR8-mediated test signal in response to a candidate immunostimulatory compound, and comparing the TLR8-mediated test signal to the TLR8-mediated reference signal.

In yet another aspect the invention provides a method for screening candidate immunostimulatory compounds, comprising measuring a TLR8-mediated reference signal in  
10 response to an imidazoquinoline reference, measuring a TLR8-mediated test signal in response to a candidate immunostimulatory compound, and comparing the TLR8-mediated test signal to the TLR8-mediated reference signal.

Also provided according to yet another aspect of the invention is a method for screening candidate immunostimulatory compounds. The method involves measuring a  
15 TLR7-mediated reference signal in response to an imidazoquinoline reference, measuring a TLR7-mediated test signal in response to a candidate immunostimulatory compound, and comparing the TLR7-mediated test signal to the TLR7-mediated reference signal.

In some embodiments the imidazoquinoline is resiquimod (R-848).

In some embodiments the imidazoquinoline is imiquimod (R-837).

20 In a further aspect the invention also provides a method for screening candidate immunostimulatory compounds. The method according to this aspect involves measuring a TLR7-mediated reference signal in response to a 7-allyl-8-oxoguanosine reference, measuring a TLR7-mediated test signal in response to a candidate immunostimulatory compound, and comparing the TLR7-mediated test signal to the TLR7-mediated reference  
25 signal.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention.

### 30 **Brief Description of the Figures**

FIG. 1 is a bar graph depicting IL-12 p40 secretion by human peripheral blood mononuclear cells (PBMCs) in response to certain stimuli including selected G,U-containing

- 16 -

RNA oligonucleotides with or without DOTAP ("with Liposomes" and "without Liposomes", respectively), as measured by specific enzyme-linked immunosorbent assay (ELISA). The lower case letter "s" appearing in the base sequences signifies phosphorothioate linkage.

5        FIG. 2 is a bar graph depicting TNF- $\alpha$  secretion by human PBMCs in response to certain stimuli including selected G,U-containing RNA oligonucleotides with or without DOTAP ("with Liposomes" and "without Liposomes", respectively), as measured by specific ELISA.

10        FIG. 3 is a bar graph depicting dose-dependence of IL-12 p40 secretion by human PBMCs in response to various concentrations of selected G,U-containing RNA oligonucleotides (with DOTAP), as measured by specific ELISA.

      FIG. 4 is a bar graph depicting sequence dependence of TNF- $\alpha$  secretion by human PBMCs in response to various selected RNA oligonucleotides related to the RNA oligonucleotide GUAGGCAC (with DOTAP), as measured by specific ELISA.

15        FIG. 5 is a bar graph depicting the effect of DOTAP on IL-12 p40 secretion by human PBMCs in response to various stimuli, as measured by specific ELISA.

      FIG. 6 is a quartet of bar graphs depicting IL-12 p40 secretion by various types of murine macrophage cells in response to a variety of test and control immunostimulatory compounds, as measured by specific ELISA. Panel A, wild type macrophages in the presence of IFN- $\gamma$ ; Panel B, MyD88-deficient macrophages in the presence of IFN- $\gamma$ ; Panel C, J774 macrophage cell line; Panel D, RAW 264.7 macrophage cell line.

20        FIG. 7 is a pair of graphs depicting the secretion of (A) TNF- $\alpha$  and (B) IL-12 p40 by human PBMC upon incubation with HIV-1-derived RNA sequences, with and without DOTAP. Circles, 5'-GUAGUGUGUG-3' (SEQ ID NO:2); Triangles, 5'-GUCUGUUGUGUG-3' (SEQ ID NO:3). Open symbols, without DOTAP; closed symbols, with DOTAP.

      FIG. 8 is a graph depicting apparent relatedness among TLRs.

      FIG. 9 depicts nucleic acid binding domains in TLR7, TLR8, and TLR9.

30        FIG. 10 is a bar graph depicting responsiveness of human PBMC to stringent response factor (SRF).

      FIG. 11 is a bar graph depicting responsiveness of human PBMC to the ribonucleoside vanadyl complexes (RVCs). X denotes resiquimod.

FIG. 12 is a series of three bar graphs depicting responsiveness of human TLR7 and human TLR8 to individual ribonucleosides. X denotes resiquimod.

FIG. 13 is a series of three bar graphs depicting responsiveness of TLR7 and TLR8 to mixtures of two ribonucleosides.

FIG. 14 is a bar graph depicting response of human PBMC to a mixture of the ribonucleosides G and U.

FIG. 15 is a bar graph depicting response of human PBMC to G,U-rich RNA, but not DNA, oligonucleotides.

FIG. 16 is a bar graph depicting response of human PBMC to oxidized RNA.

FIG. 17 is a series of three bar graphs depicting human TLR7 and TLR8 responses to oxidized guanosine ribonucleoside. X denotes resiquimod.

FIG. 18 is a pair of bar graphs depicting human TLR7 responses to modified guanosine ribonucleosides.

FIG. 19 is a series of aligned gel images depicting differential expression of TLR1-  
15 TLR9 on human CD123+ dendritic cells (CD123+ DC), CD11c+ DC, and neutrophils.

FIG. 20 is a series of three graphs depicting the ability of short, single-stranded G,U-containing RNA oligomers to induce NF- $\kappa$ B in HEK-293 cells stably transfected with expression plasmid for human TLR7 or human TLR8.

**Detailed Description of the Invention**

The invention relates in part to the discovery by the inventors of a number of RNA and RNA-related molecules that are effective as immunostimulatory compounds. Identification of the immunostimulatory compounds arose through a systematic effort aimed at identifying naturally occurring ligands for TLR7 and TLR8. As a result of this effort, it has now been discovered that RNA and RNA-like molecules containing guanine (G) and uracil (U), including specific sequences containing G and U, are immunostimulatory and appear to act through an MyD88-dependent pathway, implicating TLR involvement. Significantly, some of the RNA sequences occur in highly conserved structural features of 5' untranslated regions of viral RNA that are important to viral replication. The identified immunostimulatory RNA sequences also correspond to or very nearly correspond to other RNAs, including tRNAs derived from bacteria and yeast, as well as rRNA derived from bacteria and possibly some eukaryotes. Importantly, the immunostimulatory RNA of the

- 18 -

invention includes single-stranded RNA, in addition to partially or wholly double-stranded RNA, and its effect can be abrogated by RNase treatment. Where the RNA is at least partially double-stranded, it can in one embodiment include a stem-loop structure. As described in greater detail below, it has been discovered according to the invention that  
5 single-stranded G,U-rich RNAs as short as 5 nucleotides long can stimulate immune cells to produce large amounts of a number of cytokines and chemokines, including TNF- $\alpha$ , IL-6, IL-12, type 1 interferon (e.g., IFN- $\alpha$ ), and IP-10.

It has now been surprisingly discovered by the inventors that certain G,U-containing RNA molecules and their analogs, but not their DNA counterparts, are immunostimulatory.  
10 Significantly, the G,U-containing oligoribonucleotides of the invention can be substantially smaller than the messenger RNAs previously described to be useful in preparing dendritic cell vaccines. See, e.g., Boczkowski D et al. (1996) *J Exp Med* 184:465-72; Mitchell DA et al. (2000) *Curr Opin Mol Ther* 2:176-81. Although the G,U-containing RNA molecules of the invention can be surrogates for ribosomal RNA and/or viral RNA as found in nature, they  
15 can be as small as 5-40 nucleotides long. As described further herein, the G,U-containing oligoribonucleotides of the invention include at least one G and at least one U. Surprisingly, elimination of either G or U from the G,U-containing oligoribonucleotides of the invention essentially abrogates their immunostimulatory effect. The at least one G and at least U can be adjacent to one another, or they can be separated by intervening nucleosides or sequence.  
20 Also significantly, the immunostimulatory G,U-containing RNA molecules of the invention do not require a CpG dinucleotide.

In one aspect the invention provides an immunostimulatory composition. The immunostimulatory composition according to this aspect of the invention includes an isolated RNA oligomer 5-40 nucleotides long having a base sequence having at least one guanine (G)  
25 and at least one uracil (U). As will be described in greater detail further below, the immunostimulatory RNA oligomer 5-40 nucleotides long having a base sequence having at least one guanine (G) and at least one uracil (U) is advantageously formulated such that the RNA oligomer is stabilized against degradation, concentrated in or on a particle such as a liposome, and/or targeted for delivery to the endosomal compartment of cells. In one  
30 formulation, described in the examples below, the RNA oligomer is advantageously combined with the cationic lipid DOTAP, which is believed to assist in trafficking the G,U-containing oligoribonucleotides into the endosomal compartment. Thus, in one aspect the

- 19 -

invention is an immunostimulatory composition which includes an RNA oligomer 5-40 nucleotides long having a base sequence having at least one G and at least one U and optionally a cationic lipid.

The RNA oligomer of the invention can be of natural or non-natural origin. RNA as it occurs in nature is a type of nucleic acid that generally refers to a linear polymer of certain ribonucleoside units, each ribonucleoside unit made up of a purine or pyrimidine base and a ribose sugar, linked by internucleoside phosphodiester bonds. In this regard "linear" is meant to describe the primary structure of RNA. RNA in general can be single-stranded or double-stranded, including partially double-stranded.

As used herein, "nucleoside" refers to a single sugar moiety (e.g., ribose or deoxyribose) linked to an exchangeable organic base, which is either a substituted pyrimidine (e.g., cytosine (C), thymidine (T) or uracil (U)) or a substituted purine (e.g., adenine (A) or guanine (G)). As described herein, the nucleoside may be a naturally occurring nucleoside, a modified nucleoside, or a synthetic (artificial) nucleoside.

The terms "nucleic acid" and "oligonucleotide" are used interchangeably to mean multiple nucleotides (i.e., molecules comprising a sugar (e.g., ribose or deoxyribose) linked to a phosphate group and to an exchangeable organic base, which is either a substituted pyrimidine (e.g., cytosine (C), thymidine (T) or uracil (U)) or a substituted purine (e.g., adenine (A) or guanine (G)). As used herein, the terms refer to oligoribonucleotides as well as oligodeoxyribonucleotides. The terms shall also include polynucleosides (i.e., a polynucleotide minus the phosphate) and any other organic base-containing polymer. Nucleic acid molecules can be obtained from existing nucleic acid sources (e.g., genomic or cDNA), but are preferably synthetic (e.g., produced by nucleic acid synthesis).

The terms nucleic acid and oligonucleotide also encompass nucleic acids or oligonucleotides with substitutions or modifications, such as in the bases and/or sugars. For example, they include nucleic acids having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified nucleic acids may include a 2'-O-alkylated ribose group. In addition, modified nucleic acids may include sugars such as arabinose instead of ribose. Thus the nucleic acids may be heterogeneous in backbone composition thereby containing any possible combination of polymer units linked together such as peptide nucleic acids (which have amino acid backbone with nucleic acid bases). In

- 20 -

some embodiments, the nucleic acids are homogeneous in backbone composition. Nucleic acids also include substituted purines and pyrimidines such as C-5 propyne modified bases. Wagner RW et al. (1996) *Nat Biotechnol* 14:840-4. Purines and pyrimidines include but are not limited to adenine, cytosine, guanine, thymidine, 5-methylcytosine, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, and other naturally and non-naturally occurring nucleobases, substituted and unsubstituted aromatic moieties. Other such modifications are well known to those of skill in the art.

A natural nucleoside base can be replaced by a modified nucleoside base, wherein the modified nucleoside base is for example selected from hypoxanthine; dihydrouracil; pseudouracil; 2-thiouracil; 4-thiouracil; 5-aminouracil; 5-(C<sub>1</sub>-C<sub>6</sub>)-alkyluracil; 5-(C<sub>2</sub>-C<sub>6</sub>)-alkenyluracil; 5-(C<sub>2</sub>-C<sub>6</sub>)-alkynyluracil; 5-(hydroxymethyl)uracil; 5-chlorouracil; 5-fluorouracil; 5-bromouracil; 5-hydroxycytosine; 5-(C<sub>1</sub>-C<sub>6</sub>)-alkylcytosine; 5-(C<sub>2</sub>-C<sub>6</sub>)-alkenylcytosine; 5-(C<sub>2</sub>-C<sub>6</sub>)-alkynylcytosine; 5-chlorocytosine; 5-fluorocytosine; 5-bromocytosine; N<sup>2</sup>-dimethylguanine; 2,4-diamino-purine; 8-azapurine (including, in particular, 8-azaguanine); a substituted 7-deazapurine (including, in particular, 7-deazaguanine), including 7-deaza-7-substituted and/or 7-deaza-8-substituted purine; or other modifications of a natural nucleoside bases. This list is meant to be exemplary and is not to be interpreted to be limiting.

In particular, the at least one guanine base of the immunostimulatory G,U-containing oligoribonucleotide can be a substituted or modified guanine such as 7-deazaguanine; 8-azaguanine; 7-deaza-7-substituted guanine (such as 7-deaza-7-(C<sub>2</sub>-C<sub>6</sub>)alkynylguanine); 7-deaza-8-substituted guanine; hypoxanthine; 2,6-diaminopurine; 2-aminopurine; purine; 8-substituted guanine such as 8-hydroxyguanine; and 6-thioguanine. This list is meant to be exemplary and is not to be interpreted to be limiting.

Also in particular, the at least one uracil base of the G,U-containing oligoribonucleotide can be a substituted or modified uracil such as pseudouracil and 5-methyluracil.

For use in the instant invention, the nucleic acids of the invention can be synthesized *de novo* using any of a number of procedures well known in the art. For example, the  $\beta$ -cyanoethyl phosphoramidite method (Beaucage SL et al. (1981) *Tetrahedron Lett* 22:1859); nucleoside H-phosphonate method (Garegg et al. (1986) *Tetrahedron Lett* 27:4051-4; Froehler et al. (1986) *Nucl Acid Res* 14:5399-407; Garegg et al. (1986)

- 21 -

*Tetrahedron Lett* 27:4055-8; Gaffney et al. (1988) *Tetrahedron Lett* 29:2619-22). These chemistries can be performed by a variety of automated nucleic acid synthesizers available in the market. These nucleic acids are referred to as synthetic nucleic acids. Alternatively, T-rich and/or TG dinucleotides can be produced on a large scale in plasmids, (see Sambrook T et al., "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor laboratory Press, New York, 1989) and separated into smaller pieces or administered whole. Nucleic acids can be prepared from existing nucleic acid sequences (e.g., genomic or cDNA) using known techniques, such as those employing restriction enzymes, exonucleases or endonucleases. Nucleic acids prepared in this manner are referred to as isolated nucleic acid. An isolated nucleic acid generally refers to a nucleic acid which is separated from components which it is normally associated with in nature. As an example, an isolated nucleic acid may be one which is separated from a cell, from a nucleus, from mitochondria or from chromatin. The term "nucleic acid" encompasses both synthetic and isolated nucleic acid.

For use *in vivo*, the nucleic acids may optionally be relatively resistant to degradation (e.g., are stabilized). In some embodiments only specific portions of the nucleic acids may optionally be stabilized. A "stabilized nucleic acid molecule" shall mean a nucleic acid molecule that is relatively resistant to *in vivo* degradation (e.g., via an *exo-* or *endo-*nuclease). Stabilization can be a function of length or secondary structure. Nucleic acids that are tens to hundreds of kbs long are relatively resistant to *in vivo* degradation. For shorter nucleic acids, secondary structure can stabilize and increase their effect. For example, if the 3' end of an nucleic acid has self-complementarity to an upstream region, so that it can fold back and form a sort of stem loop structure, then the nucleic acid becomes stabilized and therefore exhibits more activity.

In certain embodiments according to this aspect of the invention, the base sequence of the RNA oligomer is at least partially self-complementary. A self-complementary sequence as used herein refers to a base sequence which, upon suitable alignment, may form intramolecular or, more typically, intermolecular basepairing between G-C, A-U, and/or G-U wobble pairs. In one embodiment the extent of self-complementarity is at least 50 percent. For example an 8-mer that is at least 50 percent self-complementary may have a sequence capable of forming 4, 5, 6, 7, or 8 G-C, A-U, and/or G-U wobble basepairs. Such basepairs may but need not necessarily involve bases located at either end of the self-complementary RNA oligomer. Where nucleic acid stabilization may be important to the RNA oligomers, it

- 22 -

may be advantageous to "clamp" together one or both ends of a double-stranded nucleic acid, either by basepairing or by any other suitable means. The degree of self-complementarity may depend on the alignment between oligomers, and such alignment may or may not include single- or multiple-nucleoside overhangs. In other embodiments, the degree of self-complementarity is at least 60 percent, at least 70 percent, at least 80 percent, at least 90 percent, or even 100 percent. The foregoing notwithstanding, it should be noted that double-strandedness is not a requirement of the RNA oligomers of the invention.

Similar considerations apply to intermolecular basepairing between RNA oligonucleotides of different base sequence. Thus where a plurality of RNA oligomers are used together, the plurality of oligomers may, but need not, include sequences which are at least partially complementary to one another. In one embodiment the plurality of oligomers includes an oligomer having a first base sequence and an oligomer having a second base sequence, wherein the first base sequence and the second base sequence are at least 50 percent complementary. For example, as between two 8-mers that are at least 50 percent complementary, they may form 4, 5, 6, 7, or 8 G-C, A-U, and/or G-U wobble basepairs. Such basepairs may but need not necessarily involve bases located at either end of the complementary RNA oligomers. The degree of complementarity may depend on the alignment between oligomers, and such alignment may or may not include single- or multiple-nucleoside overhangs. In other embodiments, the degree of complementarity is at least 60 percent, at least 70 percent, at least 80 percent, at least 90 percent, or even 100 percent.

Alternatively, nucleic acid stabilization can be accomplished via phosphate backbone modifications. Preferred stabilized nucleic acids of the instant invention have a modified backbone. It has been demonstrated that modification of the nucleic acid backbone provides enhanced activity of the nucleic acids when administered *in vivo*. One type of modified backbone is a phosphate backbone modification. Inclusion in immunostimulatory nucleic acids of at least two phosphorothioate linkages at the 5' end of the oligonucleotide and multiple (preferably five) phosphorothioate linkages at the 3' end, can in some circumstances provide maximal activity and protect the nucleic acid from degradation by intracellular exo- and endonucleases. Other modified nucleic acids include phosphodiester-modified nucleic acids, combinations of phosphodiester and phosphorothioate nucleic acids, alkylphosphonate and arylphosphonate, alkylphosphorothioate and arylphosphorothioate, methylphosphonate,

- 23 -

methylphosphorothioate, phosphorodithioate, p-ethoxy, morpholino, and combinations thereof. Nucleic acids having phosphorothioate linkages provide maximal activity and protect the nucleic acid from degradation by intracellular exo- and endo-nucleases, and combinations thereof. Each of these combinations and their particular effects on immune  
5 cells is discussed in more detail with respect to CpG nucleic acids in issued U.S. Pat. Nos. 6,207,646 and 6,239,116, the entire contents of which are hereby incorporated by reference. It is believed that these modified nucleic acids may show more stimulatory activity due to enhanced nuclease resistance, increased cellular uptake, increased protein binding, and/or altered intracellular localization.

10 Modified backbones such as phosphorothioates may be synthesized using automated techniques employing either phosphoramidate or H-phosphonate chemistries. Aryl- and alkyl-phosphonates can be made, e.g., as described in U.S. Pat. No. 4,469,863; and alkylphosphotriesters (in which the charged oxygen moiety is alkylated as described in U.S. Pat. No. 5,023,243 and European Pat. No. 092,574) can be prepared by automated solid phase  
15 synthesis using commercially available reagents. Methods for making other DNA backbone modifications and substitutions have been described. Uhlmann E et al. (1990) *Chem Rev* 90:544; Goodchild J (1990) *Bioconjugate Chem* 1:165.

Other stabilized nucleic acids include: nonionic DNA analogs, such as alkyl- and aryl-phosphates (in which the charged phosphonate oxygen is replaced by an alkyl or aryl group),  
20 phosphodiester and alkylphosphotriesters, in which the charged oxygen moiety is alkylated. Nucleic acids which contain diol, such as tetraethyleneglycol or hexaethyleneglycol, at either or both termini have also been shown to be substantially resistant to nuclease degradation.

Another class of backbone modifications include 2'-O-methylribonucleosides (2'-OMe). These types of substitutions are described extensively in the prior art and in particular  
25 with respect to their immunostimulating properties in Zhao et al. (1999) *Bioorg Med Chem Lett* 9:24:3453-8. Zhao et al. describes methods of preparing 2'-OMe modifications to nucleic acids.

The immunostimulatory G,U-containing RNA oligomers of the invention are typically about 5 to about 40 nucleotides long. Thus in certain distinct embodiments, the G,U-  
30 containing RNA oligomer can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nucleotides long. In one embodiment the G,U-containing RNA oligomer can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,

- 24 -

15, 16, 17, 18, 19, or 20 nucleotides long. In one embodiment the G,U-containing RNA oligomer can be 5, 6, 7, 8, 9, 10, 11, or 12 nucleotides long. In one embodiment the G,U-containing RNA oligomer can be 8, 9, 10, 11, or 12 nucleotides long.

For example, RNA oligomers with the following base sequences have been  
5 discovered to be useful in the compositions and practice of the invention: 5'-GUGUUUAC-3'; 5'-GUAGGCAC-3'; 5'-CUAGGCAC-3'; 5'-CUCGGCAC-3'; and 5'-GUGUUUAC-3' in combination with 5'-GUAGGCAC-3'.

Because the immunostimulatory effects of the G,U-containing RNA oligomers of the invention have been discovered to be MyD88-dependent, it is the belief of the inventors that  
10 the immunostimulatory G,U-containing RNA oligomers of the invention may interact with at least one TLR as a step in exerting their immunostimulatory effect. The immunostimulatory G,U-containing RNA oligomers of the invention may thus represent or mimic at least portions of natural ligands for the at least one TLR. Such natural ligands may include ribosomal RNA, either prokaryotic or eukaryotic, as well as certain viral RNAs. The TLR or  
15 TLRs may be TLR8, TLR7, or some yet-to-be defined TLR. Natural ligands for TLR1, TLR7, TLR8, and TLR10 have not previously been described.

The immunostimulatory RNA molecules of the invention have been discovered to occur in nature in all types of RNA, usually in association with highly conserved sequence or key structural feature. In one example, immunostimulatory RNA has been discovered to  
20 occur in the context of an internal ribosome entry site (IRES).

An IRES is a minimal cis-acting RNA element contained within a complex structural feature in the 5' untranslated region (5' UTR) of viral RNA and other mRNAs that regulates the initiation of translation of the viral genome in a cap-independent manner. Hellen CU et al. (2001) *Genes Dev* 15:1593-1612. Cap-independent initiation of viral RNA translation was  
25 first observed in picornaviruses. Jackson RJ et al. (1990) *Trends Biochem Sci* 15:477-83; Jackson RJ et al. (1995) *RNA* 1:985-1000.

In most eukaryotic cells, mRNA translation initiation commences with recruitment of the cap binding complex eukaryotic initiation factor (eIF)4F, composed of eIF4E (cap binding protein), eIF4A, and eIF4G, to the 5' capped end of the mRNA. The 40S ribosomal subunit, carrying eIF3, and the ternary initiator complex tRNA-eIF2-GTP are then recruited  
30 to the 5' end of the mRNA through interaction between eIF3 and eIF4G. The 40S subunit then scans the mRNA in a 5' to 3' direction until it encounters an appropriate start codon,

- 25 -

whereupon the anticodon of initiator methionine-tRNA is engaged, the 60S subunit joins to form an 80S ribosome, and translation commences.

Thus the significance of an IRES, at least in the context of a virus, is believed to be the ability of the IRES to confer a selective advantage to the virus over usual cap-dependent translation in the cell.

The following viruses have been reported to have IRES elements in their genome: all picornaviruses; bovine viral diarrhea virus; classic swine fever virus; cricket paralysis virus; encephalomyocarditis virus; foot-and-mouth disease virus; Friend murine leukemia virus *gag* mRNA; HCV; human immunodeficiency virus *env* mRNA; Kaposi's sarcoma-associated herpesvirus; Moloney murine leukemia virus *gag* mRNA; *Plautia stali* intestine virus; poliovirus; rhinovirus; *Rhopalosiphum padi* virus; and Rous sarcoma virus. Hellen CU et al. (2001) *Genes Dev* 15:1593-1612. This list is not intended to be limiting.

The viral proteins of hepatitis C virus (HCV) are translated from a 9.5 kb single-stranded positive sense RNA which is flanked by 5' and 3' UTRs. The highly conserved 5' UTR includes an IRES present in nt 40-370. Reynolds JE et al. (1996) *RNA* 2:867-78. The HCV 5' UTR is believed to have four major structural domains (I-IV), of which domains II and III have subdomains. Subdomain IIId includes a 27 nt stem-loop (nt 253-279) that on the basis of in vivo mutational studies has been reported to be critical in HCV IRES-mediated translation. Kieft JS et al. (1999) *J Mol Biol* 292:513-29; Klinck R et al. (2000) *RNA* 6:1423-31. The sequence of the IIId 27-mer is provided by 5'-GCCGAGUAGUGUUGGGUCGCGAAAGGC-3' (SEQ ID NO:4), wherein the UUGGU forms the terminal loop. The stem-loop structure is reported to include a number of non-Watson—Crick base pairs, typical of other RNAs, including wobble U•G, U•A, G•A, and A•A base pairs.

As another example, the immunostimulatory RNA sequences of the invention have been discovered to occur in G,U-rich sequence near the 5' end of the viral RNA of human immunodeficiency virus type 1 (HIV-1) that is crucial to efficient viral RNA packaging. Russell RS et al. (2002) *Virology* 303:152-63. Specifically, two key G,U-rich sequences within U5, namely 5'-GUAGUGUGUG-3' (SEQ ID NO:2) and 5'-GUCUGUUGUGUG-3' (SEQ ID NO:3), corresponding to nt 99-108 and 112-123 of strain BH10, respectively, have been found according to the present invention to be highly immunostimulatory (see Example

- 26 -

11 below). It will be noted that SEQ ID NO:2 includes both GUAGU and GUGUG, and SEQ ID NO:3 includes GUGUG.

As yet another example, the immunostimulatory RNA sequences of the invention have been found to occur in 5S ribosomal RNA loop E of a large number of species of  
5 bacteria.

TLR8 and TLR7 show high sequence homology to TLR9 (**FIG. 8**). TLR9 is the CpG-DNA receptor and transduces immunostimulatory signals. Two DNA binding motifs have been described in TLR9 (U.S. Pat. Application No. 09/954,987) that are also present in TLR8 and TLR7 with some modifications (**FIG. 9**). Despite this similarity, however, TLR7  
10 and TLR8 do not bind CpG-DNA.

It has been discovered according to the present invention that guanosine, particularly guanosine in combination with uracil, and certain guanosine-containing nucleic acids and derivatives thereof, are natural ligands of TLR8. It has been discovered according to the present invention that RNA, oxidized RNA, G,U-rich nucleic acids, and at least partially  
15 double-stranded nucleic acid molecules having at least one G-U base pair are TLR8 ligands. In certain preferred embodiments involving guanosine, guanosine derivatives, and G,U-rich nucleic acids, guanosine is the ribonucleoside. Nucleic acid molecules containing GUU, GUG, GGU, GGG, UGG, UGU, UUG, UUU, multiples and any combinations thereof are believed to be TLR8 ligands. In some embodiments the TLR8 ligand is a G,U-rich  
20 oligonucleotide that includes a hexamer sequence (UUGUGG)<sub>n</sub>, (UGGUUG)<sub>n</sub>, (GUGUGU)<sub>n</sub>, or (GGGUUU)<sub>n</sub> where n is an integer from 1 to 8, and preferably n is at least 3. In addition, it has also been discovered according to the present invention that mixtures of ribonucleoside vanadyl complexes (i.e., mixtures of adenine, cytosine, guanosine, and uracil ribonucleoside vanadyl complexes), and guanosine ribonucleoside vanadyl complexes alone, are TLR8  
25 ligands. In addition, it has been discovered according the present invention that certain imidazoquinolines, including resiquimod and imiquimod, are TLR8 ligands.

It has also been discovered according to the present invention that guanosine, and certain guanosine-containing nucleic acids and derivatives thereof, are natural ligands of TLR7. It has been discovered according to the present invention that RNA, oxidized RNA,  
30 G-rich nucleic acids, and at least partially double-stranded nucleic acid molecules that are rich in G content are TLR7 ligands. In certain preferred embodiments involving guanosine, guanosine derivatives, and G-rich nucleic acids, guanosine is the ribonucleoside. In addition,

- 27 -

it has also been discovered according to the present invention that mixtures of ribonucleoside vanadyl complexes (i.e., mixtures of adenine, cytosine, guanosine, and uracil ribonucleoside vanadyl complexes), and guanosine ribonucleoside vanadyl complexes alone, are TLR7 ligands. In addition, it has been discovered according to the present invention that 7-allyl-8-oxoguanosine (loxoribine) is a TLR7 ligand.

In addition to having diverse ligands, the various TLRs are believed to be differentially expressed in various tissues and on various types of immune cells. For example, human TLR7 has been reported to be expressed in placenta, lung, spleen, lymph nodes, tonsil and on plasmacytoid precursor dendritic cells (pDCs). Chuang T-H et al. (2000) *Eur Cytokine Netw* 11:372-8; Kadowaki N et al. (2001) *J Exp Med* 194:863-9. Human TLR8 has been reported to be expressed in lung, peripheral blood leukocytes (PBL), placenta, spleen, lymph nodes, and on monocytes. Kadowaki N et al. (2001) *J Exp Med* 194:863-9; Chuang T-H et al. (2000) *Eur Cytokine Netw* 11:372-8. Human TLR9 is reportedly expressed in spleen, lymph nodes, bone marrow, PBL, and on pDCs, B cells, and CD123+ DCs. Kadowaki N et al. (2001) *J Exp Med* 194:863-9; Bauer S et al. (2001) *Proc Natl Acad Sci USA* 98:9237-42; Chuang T-H et al. (2000) *Eur Cytokine Netw* 11:372-8.

Guanosine derivatives have previously been described as B-cell and NK cell activators, but their receptors and mechanism of action were not understood. Goodman MG et al. (1994) *J Pharm Exp Ther* 274:1552-57; Reitz AB et al. (1994) *J Med Chem* 37:3561-78. Such guanosine derivatives include, but are not limited to, 8-bromoguanosine, 8-oxoguanosine, 8-mercaptoguanosine, and 7-allyl-8-oxoguanosine (loxoribine).

Imidazoquinolines are synthetic small molecule immune response modifiers thought to induce expression of several cytokines including interferons (e.g., IFN- $\alpha$  and IFN- $\gamma$ ), tumor necrosis factor alpha (TNF- $\alpha$ ) and some interleukins (e.g., IL-1, IL-6 and IL-12). Imidazoquinolines are capable of stimulating a Th1 immune response, as evidenced in part by their ability to induce increases in IgG2a levels. Imidazoquinoline agents reportedly are also capable of inhibiting production of Th2 cytokines such as IL-4, IL-5, and IL-13. Some of the cytokines induced by imidazoquinolines are produced by macrophages and dendritic cells. Some species of imidazoquinolines have been reported to increase NK cell lytic activity and to stimulate B-cell proliferation and differentiation, thereby inducing antibody production and secretion.

- 28 -

As used herein, an imidazoquinoline agent includes imidazoquinoline amines, imidazopyridine amines, 6,7-fused cycloalkylimidazopyridine amines, and 1,2 bridged imidazoquinoline amines. These compounds have been described in U.S. Pat. Nos. 4689338, 4929624, 5238944, 5266575, 5268376, 5346905, 5352784, 5389640, 5395937, 5494916, 5482936, 5525612, 6039969 and 6110929. Particular species of imidazoquinoline agents include 4-amino- $\alpha,\alpha$ -dimethyl-2-ethoxymethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol (resiquimod or R-848 or S-28463; PCT/US01/28764, WO 02/22125); and 1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinoline-4-amine (imiquimod or R-837 or S-26308). Imiquimod is currently used in the topical treatment of warts such as genital and anal warts and has also been tested in the topical treatment of basal cell carcinoma.

Nucleotide and amino acid sequences of human and murine TLR3 are known. See, for example, GenBank Accession Nos. U88879, NM\_003265, NM\_126166, AF355152; and AAC34134, NP\_003256, NP\_569054, AAK26117. Human TLR3 is reported to be 904 amino acids long and to have a sequence provided in SEQ ID NO:20. A corresponding nucleotide sequence is provided as SEQ ID NO:21. Murine TLR3 is reported to be 905 amino acids long and to have a sequence as provided in SEQ ID NO:22. A corresponding nucleotide sequence is provided as SEQ ID NO:23. TLR3 polypeptide includes an extracellular domain having leucine-rich repeat region, a transmembrane domain, and an intracellular domain that includes a TIR domain.

As used herein a "TLR3 polypeptide" refers to a polypeptide including a full-length TLR3 according to one of the sequences above, orthologs, allelic variants, SNPs, variants incorporating conservative amino acid substitutions, TLR3 fusion proteins, and functional fragments of any of the foregoing. Preferred embodiments include human TLR3 polypeptides having at least 65 percent sequence identity, more preferably at least 80 percent sequence identity, even more preferably with at least 90 percent sequence identity, and most preferably with at least 95 percent sequence identity with the human TLR3 amino acid sequence of SEQ ID NO:20. Preferred embodiments also include murine TLR3 polypeptides having at least 65 percent sequence identity, more preferably at least 80 percent sequence identity, even more preferably with at least 90 percent sequence identity, and most preferably with at least 95 percent sequence identity with the murine TLR3 amino acid sequence of SEQ ID NO:22.

- 29 -

As used herein "TLR3 signaling" refers to an ability of a TLR3 polypeptide to activate the TLR/IL-1R (TIR) signaling pathway, also referred to herein as the TLR signal transduction pathway. Changes in TLR3 activity can be measured by assays such as those disclosed herein, including expression of genes under control of  $\kappa$ B-sensitive promoters and enhancers. Such naturally occurring genes include the genes encoding IL-1 $\beta$ , IL-6, IL-8, the p40 subunit of interleukin 12 (IL-12 p40), and the costimulatory molecules CD80 and CD86. Other genes can be placed under the control of such regulatory elements (see below) and thus serve to report the level of TLR3 signaling. Additional nucleotide sequence can be added to SEQ ID NO:21 or SEQ ID NO:23, preferably to the 5' or the 3' end of the open reading frame of SEQ ID NO:21, to yield a nucleotide sequence encoding a chimeric polypeptide that includes a detectable or reporter moiety, e.g., FLAG, luciferase (luc), green fluorescent protein (GFP), and others known by those skilled in the art.

SEQ ID NO:20	Human TLR3 amino acid								
15	MRQTLPCIYF	WGGLLPFGML	CASSTTKCTV	SHEVADCSHL	KLTQVPDDL	TNITVLNLTH	60		
	NQLRRLPAA	N	FTRYSQLTSL	DVGFNTISK	L	EPQLCQKLPM	LKVLNLQHNE	LSQLSDKTFA	120
	FCTNLTELHL	MSNSIQIKN	NPFVKQKNLI	TLDLSHNGLS	STKLGTQVQL	ENLQELLSN	180		
	NKIQALKSEE	LDIFANSSLK	KLELSSNQIK	EFSPGCFHAI	GRLFGFLFNN	VQLGPSLTEK	240		
	LCLELANTSI	RNLSLSNSQL	STTSNTTFLG	LKWTNLTM	LD	LSYNNLNVVG	NDSFAWLPQL	300	
20	EYFFLEYNNI	QHLFSLHSG	LFNVRYLNLK	RSFTKQSISL	ASLPKIDDFS	FQWLKCLEHL	360		
	NMEDNDIPGI	KSNMFTGLIN	LKYLSSLSNSF	TSRLTLTNET	FVSLAHSPLH	ILNLTKNKIS	420		
	KIESDAFSWL	GHLEVLDLGL	NEIGQELTQG	EWRGLENIFE	IYLSYNKYLQ	LTRNSFALVP	480		
	SLQRLMLRRV	ALKNVDSSPS	PFQPLRNLT	I	LDLSNNNIAN	INDDMLEGLE	KLEILDQLHN	540	
	NLARLWKHAN	PGGPIYFLKG	LSHLHILNLE	SNGFDEIPVE	VFKDLFELKI	IDLGLNNLNT	600		
25	LPASVFNNQV	SLKSLNLQKN	LITSVEKKVF	GPAFRNLTEL	DMRFNPFDC	T	CESIAWFVNW	660	
	INETHNIPE	LSSHLYCNTP	PHYHGFPVRL	FDTSSCKDSA	PFELFFMINT	SILLIFIFIV	720		
	LLIHFEGWRI	SFYWNVSVHR	VLGFKEIDRQ	TEQFEYAAYI	IHAYKDKDWV	WEHFSMEKE	780		
	DQSLKFCLEE	RDFAAGVFEL	EAIVNSIKRS	RKIIFVITH	LLKDPCKRF	KVHHAQQAI	840		
	EQNLDSIILV	FLEEIPDYKL	NHALCLRRGM	FKSHCILNWP	VQKERIGAFR	HKLQVALGSK	900		
30	NSVH						904		

SEQ ID NO:21	Human TLR3 nucleotide					
	cactttcgag	agtgccgtct	atttgccaca	cacttccctg	atgaaatgtc	tggatttgga 60
	ctaaagaaaa	aaggaaaggc	tagcagtcac	ccaacagaat	catgagacag	actttgcctt 120
35	gtatctactt	ttgggggggc	cttttgccct	ttgggatgct	gtgtgcatcc	tccaccacca 180
	agtgcactgt	tagccatgaa	gttgctgact	gcagccacct	gaagttgact	caggtaccgc 240
	atgatctacc	cacaaacata	acagtgttga	accttaccac	taatcaactc	agaagattac 300
	cagccgccaa	cttcacaagg	tatagccagc	taactagctt	ggatgtagga	tttaacacca 360
	tctcaaaact	ggagccagaa	ttgtgccaga	aacttcccat	gttaaaagtt	ttgaacctcc 420
40	agcacaatga	gctatctcaa	ctttctgata	aaacctttgc	cttctgcacg	aatttgactg 480
	aactccatct	catgtccaac	tcaatccaga	aaattaaaaa	taatcccttt	gtcaagcaga 540
	agaatttaat	cacattagat	ctgtctcata	atggcttgct	atctacaaaa	ttaggaactc 600
	aggttcagct	ggaaaaatctc	caagagcttc	tattatcaaa	caataaaaatt	caagcgctaa 660
	aaagtgaaga	actggatatc	tttgccaatt	catcttttaa	aaaattagag	ttgtcatcga 720
45	atcaaatata	agagttttct	ccagggtgtt	ttcacgcaat	tggagagatta	tttggcctct 780
	ttctgaacaa	tgtccagctg	gggtccagcc	ttacagagaa	gctatgtttg	gaattagcaa 840

- 30 -

	acacaagcat	tcggaatctg	tctctgagta	acagccagct	gtccaccacc	agcaatacaa	900
	ctttcttggg	actaaagtgg	acaaatctca	ctatgctcga	tctttcctac	aacaacttaa	960
	atgtgggttg	taacgattcc	tttgcttggc	ttccacaact	agaatatttc	ttcctagagt	1020
	ataataatat	acagcatttg	ttttctcact	ctttgcacgg	gcttttcaat	gtgaggtacc	1080
5	tgaatttgaa	acggtctttt	actaaacaaa	gtatttcctt	tgcttctact	cccaagattg	1140
	atgatttttc	ttttcagtg	ctaaaatgtt	tgagacacct	taacatggaa	gataatgata	1200
	ttccaggcat	aaaaagcaat	atgttcacag	gattgataaa	cctgaaatac	ttaatgttat	1260
	ccaactcctt	tacaagtttg	cgaactttga	caaatgaaac	atttgtatca	cttgctcatt	1320
	ctcccttaca	catactcaac	ctaaccaaga	ataaaatctc	aaaaatagag	agtgatgctt	1380
10	tctcttgggt	gggccacctt	gaagtacttg	acctgggcct	taatgaaatt	gggcaagaac	1440
	tcacaggcca	ggaatggaga	ggctctagaaa	atattttcga	aatctatctt	tcctacaaca	1500
	agtacctgca	gctgactagg	aactcctttg	ccttggtccc	aagccttcaa	cgactgatgc	1560
	tccgaagggt	ggcccttaaa	aatgtggata	gctctccttc	accattccag	cctcttcgta	1620
	acttgacctt	tctggatcta	agcaaccaac	acatagccaa	cataaatgat	gacatgttgg	1680
15	agggtcttga	gaaactagaa	attctcgatt	tgacagcataa	caacttagca	cggtcttggg	1740
	aacacgcaaa	ccctgggtgg	cccattttat	tcctaaaggg	tctgtctcac	ctccacatcc	1800
	tttaacttga	gtccaacggc	tttgacgaga	tcacagttga	ggctctcaag	gattttattg	1860
	aactaaagat	catcgattta	ggattgaata	atttaaacac	acttccagca	tctgtcttta	1920
	ataatcaggt	gtctctaaag	tcattgaacc	ttcagaagaa	tctcataaca	tcggttgaga	1980
20	agaagggttt	cgggccagct	ttcaggaacc	tgactgagtt	agatatgctc	tttaatccct	2040
	ttgattgcac	gtgtgaaagt	attgcctggg	ttgttaattg	gattaacgag	accataacca	2100
	acatccctga	gctgtcaagc	cactaccttt	gcaacactcc	acctcactat	catgggttcc	2160
	cagtgaagct	ttttgataca	tcattcttgc	aagacagtgc	cccccttgaa	ctctttttca	2220
	tgatcaatac	cagtatcctg	ttgattttta	tctttattgt	acttctcatc	cactttgagg	2280
25	gctggaggat	atctttttat	tggaatgttt	cagtacatcg	agttcttggg	ttcaaagaaa	2340
	tagacagaca	gacagaacag	tttgaatatg	cagcatatat	aattcatgcc	tataaagata	2400
	aggattgggt	ctgggaacat	ttctcttcaa	tggaagagga	agaccaatct	ctcaaatttt	2460
	gtctggaaga	aagggaactt	gaggcggttg	tttttgaaat	agaagcaatt	gttaacagca	2520
	tcaaaagaag	cagaaaaaat	atttttggtt	taacacacca	tctattaaaa	gaccatttat	2580
30	gcaaaagatt	caagggtacat	catgcagttc	aacaagctat	tgaacaaaat	ctggattcca	2640
	ttatatgttg	tttccttgag	gagattccag	attataaact	gaaccatgca	ctctgtttgc	2700
	gaagaggaat	gtttaaatct	cactgcatct	tgaactggcc	agttcagaaa	gaacggatag	2760
	gtgcctttcg	tcataaattg	caagtagcac	ttggatccaa	aaactctgta	cattaaattt	2820
	atttaaatat	tcaattagca	aaggagaaac	tttctcaatt	taaaaagttc	tatggcaaat	2880
35	tttaagtttc	cataaagggt	ttataatttg	ttatttcata	tttgtaaatg	attatattct	2940
	atcacaaatta	catctcttct	aggaaaatgt	gtctccttat	ttcaggccta	tttttgacaa	3000
	ttgacttaat	tttaccctaa	ataaaacata	taagcacgta	aaaaaaaaaa	aaaaaaa	3057

SEQ ID NO:22

Murine TLR3 amino acid

40	MKGCSSYLMY	SFGGLSLWI	LLVSSTNQCT	VRYNVADCSH	LKLTHIPDDL	PSNITVLNLT	60
	HNQLRRLPPT	NFTRYSQLAI	LDAGFNSISK	LEPELCQILP	LLKVLNLQHN	ELSQISDQTF	120
	VFCTNLTELD	LMSNSIHKIK	SNPFKNQKNL	IKLDLSHNGL	SSTKLGTGVQ	LENLQELLLA	180
	KNKILALRSE	ELEFLGNSSL	RKLDLSSNPL	KEFSPGCFQT	IGKLFALLLN	NAQLNPHLTE	240
	KLCWELSNST	IQNLSLANNQ	LLATSESTFS	GLKWTNLTLQ	DLSYNNLHVD	GNGSFSYLP	300
45	LRYLSLEYNN	IQRSPRSFY	GLSNLRYLSL	KRAFTKQSVS	LASHPNIDDF	SFQWLKYLEY	360
	LNMDNNIPS	TKSNTFTGLV	SLKYLSSLSK	FTSLQTLTNE	TFVSLAHSPL	LTLNLTKNHI	420
	SKIANGTFSW	LGQLRILDG	LNEIEQKLSG	QEWRLRNIF	EIYLSYNKYL	QLSTSSPALV	480
	PSLQRLMLRR	VALKNVDISP	SPFRPLRNLT	ILDLSNNNIA	NINEDLLEGL	ENLEILDFQH	540
	NNLARLWKRA	NPGGPVNFLK	GLSHLHILNL	ESNGLDEIPV	GVFKNLFELK	SINLGLNNLN	600
50	KLEPFIFDDQ	TSLSRLNLQK	NLITSVEKDV	FGPPFQNLNS	LDMRFNPFDC	TCESISWFFN	660
	WINQTHTNIF	ELSTHYLCNT	PHHYGFPPLK	LFDTSCKDS	APFELLFIIS	TSMLLVFILV	720
	VLLIHIEGWR	ISFYWNVSVH	RILGFKEIDT	QAEQFEYTAY	IIHAHKDRDW	VWEHFSMPME	780
	QDQSLKFCLE	ERDFEAGVLG	LEAIVNSIKR	SRKIFVITH	HLLKDPLCRR	FKVHHAVQQA	840
	IEQNLDISIIL	IFLQNIPIYK	LNHALCLRRG	MPKSHCILNW	PVQKERINAF	HHKLQVALGS	900
55	RNSAH						904

- 31 -

## SEQ ID NO:23 Murine TLR3 nucleotide

	tagaatatga	tacagggatt	gcaccataa	tctgggctga	atcatgaaag	ggtgttcctc	60
	ttatctaata	tactcctttg	ggggactttt	gtccctatgg	attcttctgg	tgtcttcac	120
	aaaccaatgc	actgtgagat	acaacgtagc	tgactgcagc	catttgaagc	taacacacat	180
5	acctgatgat	cttccctcta	acataacagt	gttgaatctt	actcacaacc	aactcagaag	240
	attaccacct	accaacttta	caagatacag	ccaacttgct	atcttggatg	caggatttaa	300
	ctccatttca	aaactggagc	cagaactgtg	ccaataactc	cctttgttga	aagtattgaa	360
	cctgcaacat	aatgagctct	ctcagatttc	tgatcaaacc	tttgtcttct	gcacgaacct	420
	gacagaactc	gatctaagt	ctaactcaat	acacaaaatt	aaaagcaacc	ctttcaaaaa	480
10	ccagaagaat	ctaatacaat	tagatttgtc	tcataatggg	ttatcatcta	caaagtggg	540
	aacgggggtc	caactggaga	acctccaaga	actgctctta	gcaaaaaata	aaatccttgc	600
	gttgcaagt	gaagaacttg	agtttcttgg	caattcttct	ttacgaaagt	tggacttgtc	660
	atcaaatcca	cttaaagagt	tctccccggg	gtgtttccag	acaattggca	agttattcgc	720
	cctcctcttg	aacaacgccc	aactgaaccc	ccacctcaca	gagaagcttt	gctgggaact	780
15	ttcaaacaca	agcatccaga	atctctctct	ggctaacaac	cagctgctgg	ccaccagcga	840
	gagcactttc	tctgggctga	agtggacaaa	tctcaccag	ctcgatcttt	cctacaacaa	900
	cctccatgat	gtcggcaacg	gttcttcttc	ctactccca	agcctgaggt	atctgtctct	960
	ggagtacaac	aataatacagc	gtctgtcccc	tcgctctttt	tatggactct	ccaacctgag	1020
	gtacctgagt	ttgaagcgag	catttactaa	gcaaagtgtt	tcacttgctt	cacatcccaa	1080
20	cattgacgat	ttttcctttc	aatgggttaa	atatttgga	tatctcaaca	tggatgacaa	1140
	taatattcca	agtacaaaa	gcaatacctt	cacgggattg	gtgagtctga	agtacctaag	1200
	tctttccaaa	actttcacia	gtttgcaaac	tttaacaaat	gaaacatttg	tgtcacttgc	1260
	tcattctccc	ttgtctactc	tcaacttaac	gaaaaatcac	atctcaaaaa	tagcaaatgg	1320
	tactttctct	tggttaggcc	aactcaggat	acttgatctc	ggccttaatg	aaattgaaca	1380
25	aaaactcagc	ggccagggaat	ggagaggtct	gagaaatata	tttgagatct	acctatccta	1440
	taacaaatac	ctccaactgt	ctaccagttc	ctttgcattg	gtccccagcc	ttcaaagact	1500
	gatgctcagg	aggggtggccc	ttaaaaatgt	ggatatctcc	ccttcacctt	tccgcccctc	1560
	tcgtaacctg	accattctgg	acttaagcaa	caacaacata	gccaacataa	atgaggactt	1620
	gctggagggt	cttgagaatc	tagaaatcct	ggattttcag	cacaataact	tagccaggct	1680
30	ctggaacgc	gcaaaccctg	gtgggtcccg	taatttctcg	aaggggctgt	ctcacctcca	1740
	catcttgaat	ttagagtcca	acggttaga	tgaaatccca	gtcggggttt	tcaagaactt	1800
	attcgaaacta	aagagcatca	atctaggact	gaataactta	aacaaaacttg	aaccattcat	1860
	ttttgatgac	cagacatctc	taagggtcact	gaacctccag	aagaacctca	taacatctgt	1920
	tgagaaggat	gttttcgggc	cgctttttca	aaacctgaac	agtttagata	tgcgcttcaa	1980
35	tccgttcgac	tgacagtggt	aaagtatttc	ctgggttggt	aactggatca	accagaccca	2040
	cactaatatc	tttgagctgt	ccactcacta	cctctgtaac	actccacatc	attattatgg	2100
	cttccccctg	aagcttttcg	atacatcctc	ctgtaaagac	agcggccctt	ttgaactcct	2160
	cttcaataac	agcaccagta	tgtccttggt	ttttataact	gtggtagctg	tcattcacat	2220
	cgagggtctg	aggatctctt	tttactggaa	tggttctagt	catcggtatc	ttgggttcaa	2280
40	ggaaatagac	acacaggctg	agcagtttga	atatacagcc	tacataattc	atgcccataa	2340
	agacagagac	tggttctggg	aacatttctc	cccaatggaa	gaacaagacc	aatctctcaa	2400
	attttgccta	gaagaaaggg	actttgaagc	agggcgctct	ggacttgaag	caattgttaa	2460
	tagcatcaaa	agaagccgaa	aaatcatttt	cgttatcaca	caccatttat	taaaagaccc	2520
	tctgtgcaga	agattcaagg	tacatcacgc	agttcagcaa	gctattgagc	aaaatctgga	2580
45	ttcaattata	ctgatttttc	tccagaatat	tccagattat	aaactaaacc	atgcactctg	2640
	tttgcaaga	ggaatgttta	aatctcattg	catcttgaac	tggccagttc	agaaagaacg	2700
	gataaatgcc	tttcatcata	aattgcaagt	agcacttgga	tctcggaatt	cagcacatta	2760
	aactcatttg	aagatttgga	gtcggtaaag	ggatagatcc	aatttataaa	gggccatcat	2820
	gaatctaagt	tttacttgaa	agttttgtat	atttatttat	atgtatagat	gatgatatta	2880
50	catcacaatc	caatctcagt	tttgaaatat	ttcggttat	ttcattgaca	tctgggttat	2940
	tcactccaaa	taaacacatg	ggcagttaaa	aacatcctct	attaatagat	taccatttaa	3000
	ttcttgagg	gtatcacagc	tttaaagggt	tttaaatatt	tttatataaa	taagactgag	3060
	agttttataa	atgtaatttt	ttaaaactcg	agtcttactg	tgtagctcag	aaaggcctgg	3120
	aaattaatat	attagagagt	catgtcttga	acttatttat	ctctgcctcc	ctctgtctcc	3180
55	agagtgttgc	ttttaagggc	atgtagcacc	acaccagct	atgtacgtgt	gggattttat	3240
	aatgctcatt	tttgagacgt	ttatagaata	aaagataatt	gcttttatgg	tataaggcta	3300
	cttgaggtaa						3310

- 32 -

Nucleotide and amino acid sequences of human and murine TLR7 are known. See, for example, GenBank Accession Nos. AF240467, AF245702, NM\_016562, AF334942, NM\_133211; and AAF60188, AAF78035, NP\_057646, AAL73191, AAL73192. Human TLR7 is reported to be 1049 amino acids long and to have a sequence provided in SEQ ID NO:24. A corresponding nucleotide sequence is provided as SEQ ID NO:25. Murine TLR7 is reported to be 1050 amino acids long and to have a sequence as provided in SEQ ID NO:26. A corresponding nucleotide sequence is provided as SEQ ID NO:27. TLR7 polypeptide includes an extracellular domain having leucine-rich repeat region, a transmembrane domain, and an intracellular domain that includes a TIR domain.

As used herein a "TLR7 polypeptide" refers to a polypeptide including a full-length TLR7 according to one of the sequences above, orthologs, allelic variants, SNPs, variants incorporating conservative amino acid substitutions, TLR7 fusion proteins, and functional fragments of any of the foregoing. Preferred embodiments include human TLR7 polypeptides having at least 65 percent sequence identity, more preferably at least 80 percent sequence identity, even more preferably with at least 90 percent sequence identity, and most preferably with at least 95 percent sequence identity with the human TLR7 amino acid sequence of SEQ ID NO:24. Preferred embodiments also include murine TLR7 polypeptides having at least 65 percent sequence identity, more preferably at least 80 percent sequence identity, even more preferably with at least 90 percent sequence identity, and most preferably with at least 95 percent sequence identity with the murine TLR7 amino acid sequence of SEQ ID NO:26.

As used herein "TLR7 signaling" refers to an ability of a TLR7 polypeptide to activate the TLR/IL-1R (TIR) signaling pathway, also referred to herein as the TLR signal transduction pathway. Changes in TLR7 activity can be measured by assays such as those disclosed herein, including expression of genes under control of  $\kappa$ B-sensitive promoters and enhancers. Such naturally occurring genes include the genes encoding IL-1 $\beta$ , IL-6, IL-8, the p40 subunit of interleukin 12 (IL-12 p40), and the costimulatory molecules CD80 and CD86. Other genes can be placed under the control of such regulatory elements (see below) and thus serve to report the level of TLR7 signaling. Additional nucleotide sequence can be added to SEQ ID NO:25 or SEQ ID NO:27, preferably to the 5' or the 3' end of the open reading frame of SEQ ID NO:25, to yield a nucleotide sequence encoding a chimeric polypeptide that

includes a detectable or reporter moiety, e.g., FLAG, luciferase (luc), green fluorescent protein (GFP), and others known by those skilled in the art.

# SEQ ID NO:24 Human TLR7 amino acid

5	MVFPMWTLKR	QILILFNIIL	ISKLLGARWF	PKTLPDVTLL	DVPKNHVIVD	CTDKHLTEIP	60
	GGIPTNTTNL	TLTINHIPDI	SPASFHRLDH	LVEIDFRNC	VPIPLGSKNN	MCIKRLQIKP	120
	RSFSGLTLYLK	SLYLDGNQLL	EIPQGLPPSL	QLLSLEANNI	FSIRKENLTE	LANIEILYLG	180
	QNCYYRNPY	VSYIEKDAF	LNLTKLKVLS	LKDNNTAVP	TVLPSTLTEL	YLYNNMIAKI	240
	QEDDFNNLNQ	LQILDLSGNC	PRCYNAPFPC	APCKNNSPLQ	IPVNAFDALT	ELKVLRLHSN	300
10	SLQHVPPRWF	KNINKLQELD	LSQNFLAKEI	GDAKFLHFLP	SLIQLDLSFN	FELQVYRASM	360
	NLSQAFSSLK	SLKILRIRGY	VFKELKSFNL	SPLHNLQNL	VLDLGTNFIF	IANLSMFKQF	420
	KRLKVIDLSV	NKISPSGDSS	EVGFCSNART	SVESYEPQVL	EQLHYFRYDK	YARSCRFKNK	480
	EASFMSVNES	CYKYGQTLDL	SKNSIFFVKS	SDFQHLNFL	CLNLSGNLIS	QTLNGSEFQP	540
	LAELRYLDFS	NNRLDLLHST	AFEELHKLEV	LDISSNSHYF	QSEGITHMLN	FTKNLKVLRQ	600
15	LMMNDNDISS	STSRTESESE	LRTLEFRGNH	LDVLWREGDN	RYLQLFKNLL	KLEELDLSKN	660
	SLSFLPSGVF	DGMPPNLKNL	SLAKNGLKSF	SWKKLQCLKN	LETLDLSHNQ	LTTVPERLSN	720
	CSRSRLKNLIL	KNNQIRSLTK	YFLQDAFQLR	YLDLSSNKIQ	MIQKTSFPEN	VLNKLMLLL	780
	HNRFLCTCD	AVWFVWVNH	TEVTIPYLAT	DVTCVGPAGH	KGQSVISLDL	YTCELDLTNL	840
	ILFSLSISVS	LFLMVMMTAS	HLFYFDVWYI	YHFCKAKIKG	YQRLISPDCC	YDAFIVYDTK	900
20	DPVTEWVLA	ELVAKLEDPR	EKFHNLCLLE	RDWLPGPQVL	ENLSQSIQLS	KKTVFVMTDK	960
	YAKTENFKIA	FYLHQRLMD	EKVDVILIF	LEKPFQKSKF	LQLRKRLCGS	SVLEWPTNPQ	1020
	AHPYFWQCLK	NALATDNHVA	YSQVFKETV				1049

# SEQ ID NO:25 Human TLR7 nucleotide

25	actccagata	taggatcact	ccatgccatc	aagaaagttg	atgctattgg	gcccattctca	60
	agctgatctt	ggcacctctc	atgctctgct	ctcttcaacc	agacctctac	attccattttt	120
	ggaagaagac	taaaaatggg	gtttccaatg	tggacactga	agagacaaat	tcttatcctt	180
	tttaacataa	tcctaatttc	caaactcctt	ggggctagat	ggtttcctaa	aactctggcc	240
	tgtgatgtca	ctctggatgt	tccaaagaac	catgtgatcg	tggactgcac	agacaagcat	300
30	ttgacagaaa	ttcctggagg	tattcccacg	aacaccacga	acctcaccct	caccattaac	360
	cacataccag	acatctcccc	agcgtccttt	cacagactgg	accatctggg	agagatcgat	420
	ttcagatgca	actgtgtacc	tattccactg	gggtcaaaaa	acaacatgtg	catcaagagg	480
	ctgcagatta	aaccacagaag	ctttagtggg	ctcacttatt	taaaaatcct	ttacctggat	540
	ggaaccacgc	tactagagat	accgcagggc	ctcccgccta	gcttacagct	tctcagcctt	600
35	gaggccaaca	acatcttttc	catcagaaaa	gagaactcta	cagaactggc	caacatcgaa	660
	atactctacc	tgggccaata	ctgttattat	cgaaatcctt	gttatgtttc	atattcaata	720
	gagaaagatg	ccttcctaaa	cttgacaaa	ttaaaagtgc	tctccctgaa	agataacaat	780
	gtcacagccg	tccctactgt	tttgccatct	actttaacag	aactatatct	ctacaacaac	840
	atgattgcaa	aaatccaaga	agatgatttt	aataacctca	accaattaca	aattcttgac	900
40	ctaagtggaa	attgccctcg	ttgttataat	gccccatttc	cttgtgcgcc	gtgtaaaaaa	960
	aattctcccc	tacagatccc	tgtaaatgct	tttgtatgcg	tgacagaatt	aaaagtttta	1020
	cgtctacaca	gtaactctct	tcagcatgtg	cccccaagat	ggtttaagaa	catcaacaaa	1080
	ctccaggaac	tggatctgtc	ccaaaacttc	ttggccaaa	aaattgggga	tgctaaattt	1140
	ctgcattttc	tccccagcct	catccaattg	gatctgtctt	tcaattttga	acttcagggtc	1200
45	tatcgtgcat	ctatgaatct	atcacaagca	ttttcttcac	tgaaaagcct	gaaaattctg	1260
	cggatcagag	gatatgtctt	taaagagttg	aaaagcttta	acctctcgcc	attacataat	1320
	cttcaaaaatc	ttgaagttct	tgatcttggc	actaacttta	taaaaattgc	taacctcagc	1380
	atgtttaaac	aatttaaaag	actgaaagtc	atagatcttt	cagtgaataa	aatatcacct	1440
	tcaggagatt	caagtgaagt	tggcttctgc	tcaaatgcc	gaacttctgt	agaaagttaa	1500
50	gaacccagg	tcctggaaca	attacattat	ttcagatatg	ataagtatgc	aaggatgtgc	1560
	agattcaaaa	acaaagaggc	ttctttcatg	tctgttaatg	aaagctgcta	caagtatggg	1620
	cagaccttgg	atctaagtaa	aaatagtata	ttttttgtca	agtcctctga	ttttcagcat	1680
	ctttctttcc	tcaaatgcct	gaatctgtca	ggaaatctca	ttagccaaac	tcttaatggc	1740
	agtgaattcc	aaccttttagc	agagctgaga	tatttggact	tctccaacaa	cgggtctgat	1800

- 34 -

5 ttactccatt caacagcatt tgaagagctt cacaaactgg aagttctgga tataagcagt 1860  
 aatagccatt attttcaatc agaaggaatt actcatatgc taaactttac caagaacctt 1920  
 aaggttctgc agaaactgat gatgaacgac aatgacatct cttcctccac cagcaggacc 1980  
 atggagagtg agtctcttag aactctggaa ttcagaggaa atcacttaga tgttttatgg 2040  
 10 agagaagggtg ataacagata cttacaatta ttcaagaatc tgctaaaatt agaggaatta 2100  
 gacatctcta aaaattccct aagtttcttg ccttctggag tttttgatgg tatgcctcca 2160  
 aatctaaaga atctctcttt ggccaaaaat gggctcaaat ctttcagttg gaagaaactc 2220  
 cagtgtctaa agaacctgga aactttggac ctcagccaca accaactgac cactgtccct 2280  
 gagagattat ccaactgttc cagaagcctc aagaatctga ttcttaagaa taatcaaatc 2340  
 15 aggagtctga cgaagtatct tctacaagat gccttccagt tgcgatatct ggatctcagc 2400  
 tcaataaaaa tccagatgat ccaaaagacc agcttcccag aaaatgtcct caacaatctg 2460  
 aagatgttgc ttttgcacat taatcggttt ctgtgcacct gtgatgtgtg gtgggttgtc 2520  
 tgggtgggtta accatacggg ggtgactatt ccttaccctg ccacagatgt gacttgtgtg 2580  
 20 gggccaggag cacacaaggg ccaaagtgtg atctccctgg atctgtacac ctgtgagtta 2640  
 gatctgacta acctgattct gttctcactt tccatatctg tatctctctt tctcatggtg 2700  
 atgatgacag caagtcacct ctatttctgg gatgtgtggt atatttacca tttctgtaag 2760  
 gccaaagataa aggggtatca gcgtctaata tcaccagact gttgctatga tgcctttatt 2820  
 gtgtatgaca ctaaagaccc agctgtgacc gagtgggttt tggctgagct ggtggccaaa 2880  
 ctggaagacc caagagagaa acattttaat ttatgtctcg aggaaagga ctggttacca 2940  
 25 gggcagccag tttctgaaaa cctttcccag agcatacagc ttagcaaaaa gacagtgttt 3000  
 gtgatgcagc acaagtatgc aaagtctgaa aattttaaga tagcatttta cttgtcccat 3060  
 cagaggctca tggatgaaaa agttgatgtg attatcttga tatttcttga gaagcccttt 3120  
 cagaagtcca agttcctcca gctccggaag aggtctctgt ggagtctgt ccttgagtgg 3180  
 ccaacaaacc cgcaagctca cccatacttc tggcagtgct taaagaacgc cctggccaca 3240  
 30 gacaatcatg tggcctatag tcaggtgttc aaggaaacgg tctagccctt ctttgcaaaa 3300  
 cacaactgcc tagttttacca aggagaggcc tggctgttta aattgttttc atatatatca 3360  
 caccaaaagc gtgttttgaa attcttcaag aaatgagatt gcccatattt caggggagcc 3420  
 accaacgtct gtcacaggag ttggaaagat aaggtttata taatgcatca agtcttcttt 3480  
 cttatctctc tgtgtctcta tttgcacttg agtctctcac ctcagctcct gtaaaagagt 3540  
 35 ggcaagtaaa aaacatgggg ctctgattct cctgtaattg tgataattaa atatacacac 3600  
 aatcatgaca ttgagaagaa ctgcatttct acccttaaaa agtactggtt tatacagaaa 3660  
 taggggttaa aaaaactcaa gctctctcta tatgagacca aaatgtacta gaggtagttt 3720  
 agtgaataaa aaaaccagtc agctggccgg gcatgggtgg tcatgcttgt aatcccagca 3780  
 ctttgggagg ccgaggcagg tggatcacga ggtcaggagt ttgagaccag tctggccaac 3840  
 40 atggtgaaac ccgctctgta ctaaaaatagc tgggctggtt ggtgctgtgc 3900  
 tgtaatccca gctacttggg aggtctgaggc aggagaatcg cttgaaccgg ggaggtggag 3960  
 gtggcagtg gcccagatca cggcactgca atgcagcccg ggcaacagag ctagactgtc 4020  
 tcaaaagaac aaaaaaaaaa aaacacaaaa aaactcagtc agcttcttaa ccaattgctt 4080  
 ccgtgtcatc cagggcccca ttctgtgcag attgagtggt ggcaccacac aggtgggtgc 4140  
 45 tgcttcagtg ctctctgctc ttttctcttg ggctgtctc tgggttccat agggaaacag 4200  
 taagaaagaa agacacatcc ttaccataaa tgcataatgt ccacctaaa atagaaaaat 4260  
 atttaaatga tctgccttta tcaaaagtga tttctctac ctttgataat ttactgtctt 4320  
 aaatgttttt atctgcactg caaagtactg tatccaaagt aaaatttcct catccaatat 4380  
 ctttcaaaact gttttgttaa ctaatgccat atatttgtaa gtatctgcac acttgataca 4440  
 50 gcaacgttag atggttttga tggtaaacc taaaggagga ctccaagagt gtgtatttat 4500  
 ttatagtttt atcagagatg acaattatct gaatgccaat tatatggatt cctttcattt 4560  
 tttgctggag gatgggagaa gaaaccaaag tttatagacc ttcacattga gaaagcttca 4620  
 gttttgaact tcagctatca gattcaaaaa caacagaaag aaccaagaca ttcttaagat 4680  
 gcctgtactt tcagctgggt ataaattcat gagtcaaaag attgaaacct gaccaatttg 4740  
 55 ctttatttca tggagaagat gatctacaaa ggtgtttgtg ccatttggaa aacagcgtgc 4800  
 atgtgttcaa gccttagatt ggcatgtcg tattttctc acgtgtggca atgccaaagg 4860  
 ctttacttta cctgtgagta cacactatat gaattatttc caacgtacat ttaatcaata 4920  
 agggtcacaa attcccaaat caatctctgg aataaataga gaggttaatta aattgctgga 4980  
 gccaaactatt tcacaacttc tgtaagc 5007

SEQ ID NO:26

Murine TLR7 amino acid

MVFSMWTRKR	QILIFLNMLL	VSRVFGFRWF	PKTLPCEVKV	NIPEAHVIVD	CTDKHLTEIP	60
EGIPTNTTNL	TLTINHPSI	SPDSFRRLNH	LEEIDLRCNC	VPVLLGSKAN	VCTKRLQIRP	120

- 35 -

	GSFSGLSDLK	ALYLDGNQLL	EIPQDLPSLL	HLLSLEANNI	FSITKENLTE	LVNIETLYLG	180
	QNCYRNPCN	VSYIEKDAF	LVMRNKLVLS	LKDNNVTAVP	TTLPPNLEL	LYNNIHKKI	240
	QENDFNNLNE	LQVLDLSGNC	PRCYNVPYPC	TPCENNSPLQ	IHDNAFNSLT	ELKVLRLHSN	300
	SLQHPPTWF	KNMRNLQELD	LSQNYLAREI	EEAKFLHFLP	NLVELDFSFN	YELQVYHASI	360
5	TLPHSLSSLE	NLKILRVKGY	VFKELKNSSL	SVLHLKPRLE	VLDLGTNFIK	IADLNIFKHF	420
	ENLKLIDLSV	NKISPSEESR	EVGFCPNAQT	SVDRHGQPVL	EALHYFRYDE	YARSCRFKNK	480
	EPPSFLPLNA	DCHIYGQTL	LSRNNIFFIK	PSDFQHLSFL	KCLNLSGNTI	GQTLNGSELW	540
	PLRELRYLDF	SNNRLDLLYS	TAFEELQSLE	VLDLSSNSHY	FQAEGITHML	NFTKKLRLLD	600
	KLMMNDNDIS	TSASRTMESD	SLRILEFRGN	HLVDLWRAGD	NRYLDFFKNL	FNLEVLDISR	660
10	NSLNSLPPEV	PEGMPNPNLKN	LSLAKNGLKS	FFWDRLLQLK	HLEILDLSHN	QLTKVPERLA	720
	NCSKSLTTLI	LKHNIQIRQLT	KYFLEDALQL	RYLDISSNKI	QVIQKTSFPE	NVLNLEMLV	780
	LHHNRFLCNC	DAVWFVWVWN	HTDVTIPYLA	TDVTCVGPAG	HKGQSVISLD	LYTCELDLTN	840
	LILFSVSISS	VLFLMVMTT	SHLFFWDMWY	IYYFWKAKIK	GYQHLQSMES	CYDAFIVYDT	900
	KNSAVTEWVL	QELVAKLEDP	REKHFNLCL	ERDWLPGQPV	LENLSQSIQL	SKKTVFVMTQ	960
15	KYAKTESFKM	AFYLSHQRL	DEKVDVILI	FLEKPLQKSK	FLQLRKRLCR	SSVLEWPANP	1020
	QAHYPWFQCL	KNALTTDNHV	AYSQMFKETV				1050

## SEQ ID NO:27 Murine TLR7 nucleotide

	attctcctcc	accagacctc	ttgattccat	tttgaaagaa	aactgaaaat	gggtgttttcg	60
20	atgtggacac	ggaagagaca	aattttgatc	tttttaata	tgctcttagt	ttctagagtc	120
	tttgggtttc	gatgtgttcc	taaaactcta	ccttgtagag	ttaaagtaaa	tatcccagag	180
	gcccatgtga	tcgtggactg	cacagacaag	catttgacag	aaatccctga	gggcattccc	240
	actaacacca	ccaatcttac	ccttaccatc	aaccacatac	caagcatctc	tccagattcc	300
	ttccgtaggc	tgaaccatct	ggaagaaatc	gatttaagat	gcaatttgtg	acctgttcta	360
25	ctgggggtcca	aagccaatgt	gtgtaccaag	aggctgcaga	ttagacctgg	aagctttagt	420
	ggactctctg	acttaaaagc	cctttacctg	gatggaaacc	aacttctgga	gataccacag	480
	gatctgcatc	ccagcttaca	tcttctgagc	cttgaggcta	acaacatctt	ctccatcacg	540
	aaggagaaac	taacagaact	gggtcaactt	gaaacactct	acctgggtca	aaactgttat	600
	tatcgaaatc	cttgcaatgt	ttcctattct	attgaaaaag	atgctttcct	agttatgaga	660
30	aatttgaagg	ttctctcact	aaaagataac	aatgtcacag	ctgtccccac	cactttgcca	720
	cctaattttac	tagagctcta	tctttataac	aatatcatta	agaaaatcca	agaaaatgat	780
	tttaataaacc	tcaatgagtt	gcaagttctt	gacctaaagt	gaaattgccc	tcgatgttat	840
	aatgtccccat	atccgtgtac	accgtgtgaa	aataattccc	ccttacagat	ccatgacaat	900
	gctttcaatt	cattgacaga	attaaaaagt	ttacgtttac	acagtaattc	tcttcagcat	960
35	gtgcccccaa	catgggttaa	aaacatgaga	aacctccagg	aactagacct	ctcccaaaac	1020
	tacttggcca	gagaaattga	ggaggccaaa	tttttgcat	ttcttcccaa	ccttggttgag	1080
	ttggattttt	ctttcaatta	tgagctgcag	gtctaccatg	catctataac	tttaccacat	1140
	tcactctctt	cattggaaaa	cttgaaaatt	ctgcgtgtca	aggggtatgt	ctttaaagag	1200
	ctgaaaaaact	ccagtctttc	tgtattgcac	aaagctccca	ggctggaagt	tcttgacctt	1260
40	ggcactaact	tcataaaaat	tgctgacctc	aacatattca	aacattttga	aaacctcaaa	1320
	ctcatagacc	tttcagtga	taagatatct	ccttcagaag	agtcagagag	agttggcttt	1380
	tgctcctaag	ctcaaaactc	tgtagaccgt	catgggcccc	aggctcctga	ggccttacac	1440
	tattttccgat	acgatgaata	tgacgggagc	tgacgggtca	aaaacaaaaga	gccaccttct	1500
	ttcttgcttt	tgaatgcaga	ctgccacata	tatgggcaga	ccttagactt	aagtagaaat	1560
45	aacatatttt	ttattaaacc	ttctgatttt	cagcatcttt	catttctcaa	atgcctcaac	1620
	ttatcaggaa	acaccattgg	ccaaactctt	aatggcagtg	aactctggcc	gttgagagag	1680
	ttgcggtact	tagactttct	caacaaccgg	cttgattttac	tctactcaac	agcctttgaa	1740
	gagctccagc	gtcttgaagt	tctggatcta	agtagtaaca	gccactattt	tcaagcagaa	1800
	ggaatttacc	acatgctaaa	ctttaccaag	aaattacggc	ttctggacaa	actcatgatg	1860
50	aatgataatg	acatctctac	ttcggccagc	aggaccatgg	aaagtgactc	tcttcaaat	1920
	ctggagttca	gaggcaacca	tttagatgtt	ctatggagag	ccggtgataa	cagatacttg	1980
	gacttcttca	agaatttggt	caatttagag	gtattagata	tctccagaaa	ttccctgaat	2040
	tccttgcttc	ctgaggtttt	tgagggtatg	ccgccaaatc	taaagaatct	ctccttggcc	2100
	aaaaatgggc	tcaaatcttt	cttttgggac	agactccagt	tactgaagca	tttggaaatt	2160
55	ttggacctca	gccataacca	gctgacaaaa	gtacctgaga	gattggccaa	ctgttccaaa	2220
	agtctcaca	cactgattct	taagcataat	caaatcaggc	aattgacaaa	atattttcta	2280
	gaagatgctt	tgcaattgcg	ctatctagac	atcagttcaa	ataaaatcca	ggtcattcag	2340
	aagactagct	tcccagaaaa	tgctctcaac	aatctggaga	tggttggtttt	acatcacaa	2400

- 36 -

```

cgctttcttt gcaactgtga tgctgtgtgg tttgtctggt gggttaacca tacagatggt 2460
actattccat acctggccac tgatgtgact tgtgtaggtc caggagcaca caaagggtcaa 2520
agtgtcatat cccttgatct gtatacgtgt gagttagatc tcacaaacct gattctgttc 2580
tcagtttcca tatcatcagt cctctttctt atggtagtta tgacaacaag tcacctcttt 2640
5  ttctgggata tgggttacat ttattatttt tggaaagcaa agataaagggt gtatcagcat 2700
ctgcaatcca tggagtcttg ttatgatgct tttattgtgt atgacactaa aaactcagct 2760
gtgacagaat gggttttgca ggagctggtg gcaaaattgg aagatccaag agaaaaacac 2820
ttcaatttgt gtctagaaga aagagactgg ctaccaggac agccagttct agaaaaacct 2880
tcccagagca tacagctcag caaaaagaca gtgtttgtga tgacacagaa atatgctaag 2940
10 actgagagtt ttaagatggc attttatttg tctcatcaga ggctcctgga tgaaaaagtg 3000
gatgtgatta tcttgatatt cttggaaaag cctcttcaga agtctaagtt tcttcagctc 3060
aggaagagac tctgcaggag ctctgtcctt gagtggcctg caaatccaca ggctcaccca 3120
tacttctggc agtgcctgaa aaatgccctg accacagaca atcatgtggc ttatagtcaa 3180
15 atgttcaagg aacagtccta gctctctgaa gaatgtcacc acctaggaca tgccttgaat 3240
cga

```

Nucleotide and amino acid sequences of human and murine TLR8 are known. See, for example, GenBank Accession Nos. AF246971, AF245703, NM\_016610, XM\_045706, AY035890, NM\_133212; and AAF64061, AAF78036, NP\_057694, XP\_045706, AAK62677, NP\_573475. Human TLR8 is reported to exist in at least two isoforms, one 1041 amino acids long having a sequence provided in SEQ ID NO:28, and the other 1059 amino acids long having a sequence as provided in SEQ ID NO:30. Corresponding nucleotide sequences are provided as SEQ ID NO:29 and SEQ ID NO:31, respectively. The shorter of these two isoforms is believed to be more important. Murine TLR8 is 1032 amino acids long and has a sequence as provided in SEQ ID NO:32. The corresponding nucleotide sequence is provided as SEQ ID NO:33. TLR8 polypeptide includes an extracellular domain having leucine-rich repeat region, a transmembrane domain, and an intracellular domain that includes a TIR domain.

As used herein a "TLR8 polypeptide" refers to a polypeptide including a full-length TLR8 according to one of the sequences above, orthologs, allelic variants, SNPs, variants incorporating conservative amino acid substitutions, TLR8 fusion proteins, and functional fragments of any of the foregoing. Preferred embodiments include human TLR8 polypeptides having at least 65 percent sequence identity, more preferably at least 80 percent sequence identity, even more preferably with at least 90 percent sequence identity, and most preferably with at least 95 percent sequence identity with the human TLR8 amino acid sequence of SEQ ID NO:28. Preferred embodiments also include murine TLR8 polypeptides having at least 65 percent sequence identity, more preferably at least 80 percent sequence identity, even more preferably with at least 90 percent sequence identity, and most preferably

- 37 -

with at least 95 percent sequence identity with the murine TLR8 amino acid sequence of SEQ ID NO:32.

As used herein "TLR8 signaling" refers to an ability of a TLR8 polypeptide to activate the TLR/IL-1R (TIR) signaling pathway, also referred to herein as the TLR signal transduction pathway. Changes in TLR8 activity can be measured by assays such as those disclosed herein, including expression of genes under control of  $\kappa$ B-sensitive promoters and enhancers. Such naturally occurring genes include the genes encoding IL-1 $\beta$ , IL-6, IL-8, the p40 subunit of interleukin 12 (IL-12 p40), and the costimulatory molecules CD80 and CD86. Other genes can be placed under the control of such regulatory elements (see below) and thus serve to report the level of TLR8 signaling. Additional nucleotide sequence can be added to SEQ ID NO:29 or SEQ ID NO:33, preferably to the 5' or the 3' end of the open reading frame of SEQ ID NO:29, to yield a nucleotide sequence encoding a chimeric polypeptide that includes a detectable or reporter moiety, e.g., FLAG, luciferase (luc), green fluorescent protein (GFP), and others known by those skilled in the art.

# SEQ ID NO:28 Human TLR8 amino acid (1041)

	MENMFLQSSM	LTCIFLLISG	SCELCAEENF	SRSYPCDEKK	QNDSVIAECS	NRRLQEVPT	60
	VGKYVTELDL	SDNFITHITN	ESFQGLQNL	KINLNHNPNV	QHONGNPGIQ	SNGLNITDGA	120
	FLNLKNLREL	LLEDNQLPQI	PSGLPESL	LSLIQNNIYN	ITKEGISRLI	NLKNLYLAWN	180
20	CYFNKVCEKT	NIEDGVFETL	TNLELLSLSF	NSLSHVPPKL	PSSLRKLFLS	NTQIKYISEE	240
	DFKGLINLTL	LDLSGNCPRC	FNAPFPCVPC	DGGASINIDR	FAFQNLTLQR	YLNLSSTSLR	300
	KINAAWFKNM	PHLKVLDLEF	NYLVGEIASG	AFLTMLPRLE	ILDLSFNYIK	GSYPQHINIS	360
	RNFSKLLSLR	ALHLRGYVFQ	ELREDDFQPL	MQLPNLSTIN	LGINFIKQID	FKLRFQNFNL	420
	EIIYLSENRI	SPLVKDTRQS	YANSSSFQRH	IRKRRSTDFE	FDPHSNFIYHF	TRPLIKPQCA	480
25	AYGKALDLSL	NSIFFIGPNQ	FENLPDIACL	NLSANSNAQV	LSGTEFSAIP	HVKYLDLTNN	540
	RLDFDNASAL	TELSDEVL	LSYNSHYFRI	AGVTHHLEFI	QNFTNLKVLN	LSHNNIYTLT	600
	DKYNLESKSL	VELVFSGNRL	DILWNDDNDR	YISIFKGLKN	LTRLDSLNR	LKHIPNEAFL	660
	NLPASLTSLH	INDNMLKFFN	WTLLQQFPR	ELLDLRGNKL	LFLTDSLSD	TSSRLTLLS	720
	HNRISHLPSG	FLSEVSSLKH	LDLSSNLLKT	INKSALETKT	TKLSMLELH	GNPFECTCDI	780
30	GDFRRWMDH	LNKIPRLVD	VICASPGDQR	GKSIVSLELT	TCVSDVTAVI	LFFFTFFITT	840
	MVMLAALAH	LFYWDVWFIY	NVCLAKVKGY	RSLSTSQT	DAYISYDTKD	ASVTDWVINE	900
	LYRHLEESRD	KNVLLCLEER	DWDPLAII	NLMQSINQSK	KTVFVLTKKY	AKSWNFKTAF	960
	YLALQRLMDE	NMDVIIIFILL	EPVLQHSQYL	RLRQRICKSS	ILQWPDNPKA	EGLFWQTLRN	1020
	VVLTENDSRY	NNMYVDSIKQ	Y				1041

# SEQ ID NO:29 Human TLR8 nucleotide

	ttctgcgctg	ctgcaagtta	cggaaatgaaa	aattagaaca	acagaaacat	ggaaaacatg	60
	ttccttcagt	cgtcaatgct	gacctgcatt	ttcctgctaa	tatctggttc	ctgtgagtta	120
	tgcgcggaag	aaaatttttc	tagaagctat	ccttgatgatg	agaaaaagca	aatgactca	180
40	gttattgcag	agtgcagcaa	tcgtcgacta	caggaaagttc	cccaaacggt	gggcaaatat	240
	gtgacagAAC	tagacctgtc	tgataatttc	atcacacaca	taacgaatga	atcatttcaa	300
	gggctgcaaa	atctcactaa	aataaatcta	aaccacaacc	ccaatgtaca	gcaccagaac	360
	ggaaatcccc	gtatacaatc	aaatggcttg	aatatcacag	acggggcatt	cctcaacctta	420
	aaaaaccta	gggagttact	gcttgaagac	aaccagttac	cccaaatacc	ctctggtttg	480

- 38 -

	ccagagtctt	tgacagaact	tagtctaatt	caaaacaata	tataacaacat	aactaaagag	540
	ggcattttcaa	gactttataaa	cttgaaaaat	ctctattttgg	cctggaactg	ctatttttaac	600
	aaagtttgcg	agaaaactaa	catagaagat	ggagttatttg	aaacgctgac	aaatttggag	660
	ttgctatcac	tatcttttcaa	ttctcttttca	cacgtgccac	ccaaactgcc	aagctcccta	720
5	cgcaaacttt	ttctgagcaa	caccagatc	aaatacatta	gtgaagaaga	tttcaaggga	780
	ttgataaatt	taacattact	agatttaagc	gggaactgtc	cgagggtgctt	caatgcccc	840
	tttccatgcg	tgccttgtag	tgggtggtgct	tcaatttaata	tagatcgttt	tgctttttcaa	900
	aacttgaccc	aacttcgata	cctaaacctc	tctagcactt	ccctcaggaa	gattaatgct	960
	gcctggttta	aaaatatgcc	tcatctgaag	gtgctggatc	ttgaattcaa	ctatttagtg	1020
10	ggagaaatag	cctctggggc	atttttaacg	atgctgcccc	gcttagaaat	acttgacttg	1080
	tctttttaact	atataaagg	gagttatcca	cagcatatta	atatttccag	aaactttctt	1140
	aaacttttgt	ctctacgggc	attgcattta	agaggttatg	tgttccagga	actcagagaa	1200
	gatgatttcc	agccccctgat	gcagcttcca	aacttatcga	ctatcaactt	gggtattaat	1260
	tttattaagc	aaatcgattt	caaaactttt	ccaaacttga	aaatttttac	aaatttttac	1320
15	ttgtcagaaa	acagaatata	accgttggtg	aaagataccc	ggcagagtta	tgcaaatagt	1380
	tcctcttttc	aacgtcatat	ccggaaacga	cgtcacaacg	attttgagtt	tgaccacat	1440
	tcgaactttt	atcattttcac	ccgtccttta	ataaagccac	aatgtgctgc	ttatggaaaa	1500
	gccttagatt	taagcctcaa	cagtattttc	ttcattgggc	caaaccaatt	tgaaaatctt	1560
	cctgacattg	cctgtttaaa	tctgtctgca	aatagcaatg	ctcaagtgtt	aagtggaaact	1620
20	gaatttttcag	ccatttctca	tgtcaaatat	ttggatttga	caaacaatag	actagacttt	1680
	gataatgcta	gtgctcttac	tgaattgtcc	gacttggaa	ttctagatct	cagctataat	1740
	tcacactatt	tcagaatagc	aggcgtaaca	catcatctag	aatattattca	aaatttcaca	1800
	aatctaaaag	ttttaaactt	gagccacaac	aacattttata	ctttaacaga	taagtataac	1860
	ctggaaagca	agtccctggt	agaattagtt	ttcagtggca	atcgcttga	cattttgttg	1920
25	aatgatgatg	acaacaggta	tatctccatt	ttcaaagggtc	tcaagaatct	gacacgtctg	1980
	gattttatccc	ttaataggct	gaagcacatc	ccaaatgaag	cattccctta	tttgccagcg	2040
	agtctcactg	aactacatat	aaatgataat	atgttaaagt	tttttaactg	gacattactc	2100
	cagcagttcc	ctcgtctcga	gttgcttgac	ttacgtggaa	acaaactact	ctttttaact	2160
	gatagcctat	ctctccctt	atcttccctt	cggacactgc	tgctgagtca	taacaggatt	2220
30	ttccacctac	cctctggcct	tctttctgaa	gtcagtagtc	tgaagcacct	cgatttaagt	2280
	ttcaatctgc	taaaaacaat	caacaaatcc	gcacttgaaa	ctaagaccac	caccaaatta	2340
	tctatgttgg	aactacacgg	aaaccccttt	gaatgcacct	gtgacattgg	agatttccga	2400
	agatggatgg	atgaacatct	gaatgtcaaa	attcccagac	tggtagatgt	catttgtgcc	2460
	agtcctgggg	atcaaagagg	gaagagtatt	gtgagctctg	agctgacaac	ttgtgtttca	2520
35	gatgtcactg	cagtgatatt	atttttcttc	acgttcttta	tcaccaccat	ggttatgttg	2580
	gctgccctgg	ctcaccattt	gttttactgg	gatgtttggt	ttatatataa	tgtgtgttta	2640
	gctaaggtaa	aaggctacag	gtctctttcc	acatcccaaa	ctttctatga	tgcttacatt	2700
	tcttatgaca	ccaaagatgc	ctctgttact	gactgggtga	taaatgagct	gcgtaccac	2760
	cttgaagaga	gccgagacaa	aaacgttctc	ctttgtctag	aggagaggga	ttgggacccg	2820
40	ggattggcca	tcatcgacaa	cctcatgcag	agcatcaacc	aaagcaagaa	aacagtattt	2880
	gttttaacca	aaaaatatgc	aaaaagctgg	aactttaaaa	cagcttttta	cttggttttg	2940
	cagaggctaa	tggatgagaa	catggatgtg	attatatatta	tcctgctgga	gccagtgtta	3000
	cagcattctc	agtatttgag	gctacggcag	cggatctgta	agagctccat	cctccagttg	3060
	cctgacaacc	cgaaggcaga	aggcttgttt	tggaactc	tgagaaatgt	ggtcttgact	3120
45	gaaaatgatt	cacggtataa	caatatgtat	gtcgattcca	ttaagcaata	ctaactgacg	3180
	ttaatgcatg	atttcgcgcc	ataataaaga	tgcaaaggaa	tgacatttct	gtattagtta	3240
	tctattgcta	tgtaacaaat	tatcccaaaa	cttagtggtt	taaaacaaca	catttgctgg	3300
	cccacagttt	t					3311

## 50 SEQ ID NO:30 Human TLR8 amino acid (1059)

	MKESSLQNSS	CSLGKETKKE	NMFLQSSMLT	CIFLLISGSC	ELCAEENFSR	SYPCDEKKQN	60
	DSVIAECSNR	RLQVEPQTVG	KYVTELDLSD	NFITHITNES	FQGLQNLTKI	NLNHNPNVQH	120
	QNGNPGIQSN	GLNITDGAFL	NLKNLRELL	EDNQLPQIPS	GLPESLTEL	LIQNNIYNIT	180
	KEGISRLINL	KNLYLAWNCY	FNKVCEKTN	EDGVFETLTN	LELLSLSFNS	LSHVSPKLPS	240
55	SLRKLFLSNT	QIKYISEEDF	KGLINLTLLD	LSGNCPRCFN	APFPCVPCDG	GASINIDRFA	300
	FQNLTLRLYL	NLSSTSLRKI	NAAWFKNMPH	LKVLDFEFNY	LVGEIASGAF	LTMLPRLEIL	360
	DLSFNYIKGS	YPQHINISRN	FSKPLSLRAL	HLRGYVFQEL	REDDFQPLMQ	LPNLSTINLG	420
	INFIKQIDFK	LFQNFSLNLEI	IYLSENRISP	LVKDTRQSYA	NSSSFQRHIR	KRRSTDFEFD	480

- 39 -

	PHSNFYHFTR	PLIKPQCAAY	GKALDLSLNS	IFFIGPNQFE	NLPDIACLNL	SANSNAQVLS	540
	GTEFSAIPHV	KYLDLTNNRL	DFDNASALTE	LSDLEVLDSL	YNSHYFRIAG	VTHHLEFIQN	600
	FTNLKVLNLS	HNNIYTLTDK	YNLESKSLVE	LVFSGNRLDI	LWNDDDNRYI	SIFKGLKNLT	660
	RLDLSLNRK	HIPNEAFLNL	PASLTEHLIN	DNMLKFFNWT	LLQQFPRLEL	LDLRGNKLLF	720
5	LTDLSLSDFTS	SLRTLILLSHN	RISHLPSGFL	SEVSSLKHL	LSSNLLKTIN	KSALETKTTT	780
	KLSMLELHGN	PFECTCDIGD	FRRWMDEHLN	VKIPRLVDVI	CASPGDQRGK	SIVSLELTTC	840
	VSDVTAVILF	FFTFITTMV	MLAALAHHLF	YWDVWFIYNV	CLAKIKGYRS	LSTSQTIFYDA	900
	YISYDTKDAS	VTDWVINELR	YHLEESRDKN	VLLCLEERDW	DPGLAIIDNL	MQSINQSKKT	960
	VFVLTKKYAK	SWNFKTAFYL	ALQRLMDENM	DVIIFILLEP	VLQHSQYLRL	RQRICKSSIL	1020
10	QWPDNPKAEG	LFWQTLRNVV	LTENDSRYN	MYVDSIKQY			1059

## SEQ ID NO:31 Human TLR8 nucleotide

	ctcctgcata	gagggtacca	ttctgcgctg	ctgcaagtta	cggaatgaaa	aattagaaca	60
	acagaaacgt	ggttctcttg	acacttcagt	gttagggaac	atcagcaaga	cccatcccag	120
15	gagaccttga	aggaaagcctt	tgaaaggagg	aatgaaggag	tcactcttgc	aaaatagctc	180
	ctgcagcctg	ggaaaggaga	ctaaaaagga	aaacatgttc	cttcagtcgt	caatgctgac	240
	ctgcattttc	ctgctaatat	ctgggttctg	tgagttatgc	gccgaagaaa	atttttctag	300
	aagctatcct	tgtgatgaga	aaaagcaaaa	tgactcagtt	attgcagagt	gcagcaatcg	360
	tcgactacag	gaagttcccc	aaacggtggg	caaatatgtg	acagaactag	acctgtctga	420
20	taatttcatac	acacacataa	cgaatgaatc	atttcaaggg	ctgcaaaatc	tcactaaaat	480
	aaatctaacc	cacaacccca	atgtacagca	ccagaacgga	aatcccggta	tacaatcaaa	540
	tggcttgaat	atcacagacg	gggcattcct	caacctaaaa	aacctaaagg	agttactgct	600
	tgaagacaac	cagttacccc	aaataccctc	tggtttgcc	gagtccttga	cagaacttag	660
	tctaattcaa	aacaatatat	acaacataac	taaagagggc	atttcaagac	ttataaactt	720
25	gaaaaatctc	tatttggcct	ggaactgcta	ttttaacaaa	gtttgcgaga	aaactaacat	780
	agaagatgga	gtatttgaaa	cgctgacaaa	tttgaggttg	ctatcactat	ctttcaattc	840
	tctttcacac	gtgtcaccca	aactgccaa	ctccctacgc	aaactttttc	tgagcaaac	900
	ccagatcaaa	tacattagtg	agaagattt	caagggattg	ataaatttaa	cattactaga	960
	tttaagcggg	aactgtccga	gggtcttcaa	tgccccattt	ccatgcgtgc	cttgtgtagg	1020
30	tggtgcttca	attaatatag	atcgttttgc	ttttcaaaac	ttgacccaac	ttcgatacct	1080
	aaacctctct	agcacttccc	tcaggaagat	taatgctgcc	tggtttaaaa	atatgcctca	1140
	tctgaagggtg	ctggatcttg	aattcaacta	tttagtgagg	gaaatagcct	ctggggcatt	1200
	tttaacgatg	ctgccccgct	tagaaatact	tgacttgtct	tttaactata	taaaggggag	1260
	ttatccacag	catattaata	tttccagaaa	cttctctaaa	cctttgtctc	tacgggcatt	1320
35	gcatttaaga	ggttatgtgt	tccaggaact	cagagaagat	gatttccagc	ccctgatgca	1380
	gcttccaaac	tatcgacta	tcaacttggg	tattaatttt	attaagcaaa	tcgatttcaa	1440
	acttttccaa	aattttctca	atctggaaat	tatttacttg	tcagaaaaca	gaatatcacc	1500
	gttggtaaaa	gatacccggc	agagttatgc	aaatagttcc	tcttttcaac	gtcatatccg	1560
	gaaacgacgc	tcaacagatt	ttgagtttga	cccacattcg	aacttttatc	atttcacccg	1620
40	tcctttaata	aagccacaat	gtgctgctta	tggaaaagcc	ttagatttaa	gcctcaacag	1680
	tattttcttc	attggggcaa	accaatttga	aaatcttcc	gacattgcct	gtttaaatct	1740
	gtctgcaaat	agcaatgctc	aagtgttaag	tggaaactgaa	ttttcagcca	ttcctcatgt	1800
	caaatatttg	gatttgacaa	acaatagact	agactttgat	aatgctagtg	ctcttactga	1860
	attgtccgac	ttggaagttc	tagatctcag	ctataattca	cactatttca	gaatagcagg	1920
45	cgtaacacat	catctagaat	ttattcaaaa	tttcacaaat	ctaaaagttt	taaacttgag	1980
	ccacaacaac	atttatactt	taacagataa	gtataaacctg	gaaagcaagt	ccctggtaga	2040
	attagttttc	agtggcaatc	gccttgacat	tttgtggaat	gatgatgaca	acaggtatat	2100
	ctccattttc	aaaggtctca	agaatctgac	acgtctggat	ttatccctta	ataggctgaa	2160
	gcacatccca	aatgaagcat	tccttaattt	gccagcgagt	ctcactgaac	tacatataaa	2220
50	tgataaatatg	ttaaaagttt	ttacttgagc	attactccag	cagtttcttc	gtctcagatt	2280
	gcttgactta	cgtggaaaaca	aactactctt	tttaactgat	agcctatctg	actttacatc	2340
	ttcccttcgg	acactgctgc	tgagtcataa	caggatttcc	cacctacctc	ctggctttct	2400
	ttctgaagtc	agtagtctga	agcacctcga	tttaagttcc	aatctgctaa	aaacaatcaa	2460
	caaatccgca	cttgaaacta	agaccaccac	caaattatct	atgttggaac	tacacggaaa	2520
55	cccccttgaa	tgcacctgtg	acattggaga	tttccgaaga	tggatggatg	aacatctgaa	2580
	tgtcaaaaat	ccgagactgg	tagatgtcat	ttgtgccagt	cctggggatc	aaagagggaa	2640
	gagtattgtg	agctcggagc	taacaacttg	tgtttcagat	gtcactgcag	tgatattatt	2700
	ttctctcagc	ttctttatca	ccaccatggg	tatgttggct	gccctggctc	accattttgtt	2760

- 40 -

	ttactgggat	gtttggttta	tatataatgt	gtgttttagct	aagataaaaag	gctacagggtc	2820
	tctttccaca	tcccaaactt	tctatgatgc	ttacatttct	tatgacacca	aagatgcctc	2880
	tgttactgac	tgggtgataa	atgagctgcg	ctaccacctt	gaagagagcc	gagacaaaaa	2940
	cgttctcctt	tgtctagagg	agagggattg	ggaccccgga	ttggccatca	tgcacaacct	3000
5	catgcagagc	atcaacaaaa	gcaagaaaac	agtatttgtt	ttaacaaaaa	aatatgcaaa	3060
	aagctggaac	tttaaaacag	ctttttactt	ggctttgcag	aggctaattg	atgagaacct	3120
	ggatgtgatt	atattttatc	tgctggagcc	agtgtttacag	cattctcagt	atttgaggct	3180
	acggcagcgg	atctgtaaga	gctccatcct	ccagtgccct	gacaaccgga	aggcagaagg	3240
	cttggttttg	caaaactctga	gaaatgtggt	cttgactgaa	aatgattcac	ggtataacaa	3300
10	tatgtatgtc	gattccatta	agcaatacta	actgacgtta	agtcatgatt	tgcgcgcata	3360
	ataaaga						3367

## SEQ ID NO:32 Murine TLR8 amino acid

	MENMPPQSWI	LTCFCLSSG	TSAIFHKANY	SRSYPCDEIR	HNSLVIAECN	HRQLHEVPQT	60
15	IGKYVTNIDL	SDNAITHITK	ESFQKLQNL	KIDLNHNAKQ	QHPNENKNGM	NITEGALLSL	120
	RNLTVLLED	NQLYTIPAGL	PESLKELSLI	QNNIFQVTKN	NTFGLRNLER	LYLGWNCYFK	180
	CNQTFKVEDG	AFNCLIHLKV	LSLSFNLFY	VPPKLPSLR	KLFLSNAKIM	NITQEDFKGL	240
	ENLTLLDLG	NPCRCYNAPF	PCTPKENSS	IHTPLAFQS	LTQLLYLNL	STSLRTIPST	300
	WFENLSNLKE	LHLEFNVLQ	EIASGAFLTK	LPSLQILDLS	FNFOYKEYLQ	FINISSNFSK	360
20	LRSLKKLHLR	GYVFRELKKK	HFEHLQSLPN	LATINLGINF	IEKIDFKAFQ	NFSKLDVIYL	420
	SGNRIASVLD	GTDYSSWRNR	LRKPLSTDDD	EFDPHVNFYH	STKPLIKPQC	TAYGKALDLS	480
	LNNIFIIGKS	QFEGFDIAC	LNLSFNANTQ	VFNGTEFSSM	PHIKYLDLTN	NRLDFDDMNA	540
	FSDLHDLEVL	DLSHNAHYFS	IAGVTHRLGF	IQNLINLRVL	NLSHNGIYTL	TEESELKSIS	600
	LKELVFSGNR	LDHLWNANDG	KYWSIFKSLQ	NLRLDLSYN	NLQIPNGAF	LNLPGQSLQEL	660
25	LISGNKLRF	LHLLDLSRNE	LYFLPNCLSK	FAHSLETLL	SHNHFSHLPS		720
	GFLSEARNLV	HLDFSNTIK	MINKSSLQTK	MKTNLSILEL	HGNYFDCTCD	ISDFRSWLDE	780
	NLNTIPKLV	NVICSNPGDQ	KSKSIMSLDL	TTCVSDTTAA	VLFFLTFLT	SMVMLAALVH	840
	HLFYWDVWFI	YHMCASAKLG	YRTSSTSQTF	YDAYISYDTK	DASVTDWVIN	ELRYHLEESE	900
	DKSVLLCLEE	RDWDPLPII	DNLMQSINQS	KKTIFVLTKK	YAKSWNFKTA	FYLALQRLMD	960
30	ENMDVIIIFIL	LEPVLQYSQY	LRLRQRICKS	SILQWPNNPK	AENLFWQSLK	NVVLTEENDSR	1020
	YDDLIDISIR	QY					1032

## SEQ ID NO:33 Murine TLR8 nucleotide

	attcagagtt	ggatgttaag	agagaaacaa	acgttttacc	ttcctttgtc	tatagaacat	60
35	ggaaaacatg	ccccctcagt	catggattct	gacgtgcttt	tgtctgctgt	cctctggaac	120
	cagtgccatc	ttccataaag	cgaactattc	cagaagctat	ccttgtagacg	agataaggca	180
	caactccctt	gtgattgcag	aatgcaacca	tcgtcaactg	catgaagttc	cccaactat	240
	aggcaagtat	gtgacaaaca	tagacttgct	agacaatgcc	attacacata	taacgaaaga	300
	gtcctttcaa	aagctgcaaa	acctcactaa	aatcgatctg	aaccacaatg	ccaaacaaca	360
40	gcacccaaat	gaaaataaaa	atggatatgaa	tattacagaa	ggggcacttc	tcagcctaag	420
	aaatctaaca	gttttactgc	tggaagacaa	ccagttatat	actatacctg	ctgggttgcc	480
	tgagtctttg	aaagaactta	gcctaattca	aaacaatata	tttcaggtaa	ctaaaaacaa	540
	cacttttggg	cttaggaact	tggaagact	ctatttgggc	tggaactgct	attttaaatg	600
	taatcaaac	tttaaggtag	aagatggggc	atttaaaaat	cttatacact	tgaaggtag	660
45	ctcattatct	ttcaataaac	ttttctatgt	gcccccaaa	ctaccaagtt	ctctaaggaa	720
	actttttctg	agtaatgcca	aatcatgaa	catcactcag	gaagacttca	aaggactgga	780
	aaatttaaca	ttactagatc	tgagtggaaa	ctgtccaagg	tggtacaatg	ctccatttcc	840
	ttgcacacct	tgcaaggaaa	actcatccat	ccacatacat	cctctggctt	ttcaagttc	900
	cacccaactt	ctctatctaa	acctttccag	cattccctc	aggacgattc	cttctacctg	960
50	gtttgaaaat	ctgtcaaatc	tgaaggaaact	ccatcttgaa	ttcaactatt	tagttcaaga	1020
	aattgcctcg	ggggcatttt	taacaaaact	acccagtta	caaactcctg	atttgtcctt	1080
	caactttcaa	tataaggaat	atttacaatt	tattaatatt	tcctcaaatt	tctctaagct	1140
	tcgttctctc	aagaagttgc	acttaagagg	ctatgtgttc	cgagaactta	aaaagaagca	1200
	tttcgagcat	ctccagagtc	ttccaaactt	ggcaaccatc	aacttgggca	ttacttttat	1260
55	tgagaaaatt	gatttcaaag	ctttccagaa	tttttccaaa	ctcgacgtta	tctattttatc	1320
	aggaaatcgc	atagcatctg	tattagatgg	tacagattat	tcctcttggc	gaaatcgtct	1380

- 41 -

```

5   tcggaacct ctctcaacag acgatgatga gtttgatcca cacgtgaatt tttaccatag 1440
    caccaaact ttaataaagc cacagtgtac tgcttatggc aaggccttgg atttaagttt 1500
    gaacaatatt ttcatatttg ggaaaagcca atttgaaggt tttcaggata tcgcctgctt 1560
    aaatctgtcc ttcaatgcca atactcaagt gtttaatggc acagaattct cctccatgcc 1620
10  ccacattaaa tatttggatt taaccaacaa cagactagac tttgatgata acaatgcttt 1680
    cagtgatctt cagcatctag aagtgtctga cctgagccac aatgcacact atttcagtat 1740
    agcaggggta acgcaccgtc taggatttat ccagaactta ataaacctca ggggtgtttaa 1800
    cctgagccac aatggcattt acaccctcac agaggaaagt gagctgaaaa gcactctcact 1860
    gaaagaattg gttttcagtg gaaatcgctc tgaccatttg tggaaatgcaa atgatggcaa 1920
15  atactggctc atttttataa gtctccagaa tttgatacgc ctggacttat catacaataa 1980
    ccttcaacaa atcccaaatg gagcattctc caatttgcct cagagcctcc aagagttact 2040
    tatcagggtt aacaaattac gtttctttaa ttggacatta ctccagtatt ttctcacct 2100
    tcaattgtcg gatttatcga gaaatgagct gtattttcta cccaattgcc tatctaagtt 2160
    tgcacattcc ctggagacac tgctactgag ccataatcat ttctctcacc taccctctgg 2220
15  cttctctctc gaagccagga atctggtgca cctggatcta agtttcaaca caataaagat 2280
    gatcaataaa tcctccctgc aaaccaagat gaaaacgaac ttgtctattc tggagctaca 2340
    tgggaactat tttgactgca cgtgtgacat aagtgatttt cgaagctggc tagatgaaaa 2400
    tctgaatatc acaattccta aattggtaaa tgttatatgt tccaatcctg gggatcaaaa 2460
    atcaaagagt atcatgagcc tagatctcac gacttgtgta tcggatacca ctgcagctgt 2520
20  cctgtttttc ctacatttcc ttaccacctc catggttatg ttggctgtct tggttcacca 2580
    cctgtttttc tgggatgttt ggtttatcta tcacatgtgc tctgctaagt taaaaggcta 2640
    caggacttca tccacatccc aaactttcta tgatgcttat atttcttatg acaccaaaga 2700
    tgcattctgt actgactggg taatcaatga actgcgctac caccttgaag agagtgaaga 2760
    caaaagtgtc ctcttttgtt tagaggagag ggattgggat ccaggattac ccatcattga 2820
25  taacctcatg cagagcataa accagagcaa gaaaacaatc tttgttttaa ccaagaaata 2880
    tgccaagagc tggaaactta aaacagcttt ctacttgcc ttgcagaggc taatggatga 2940
    gaacatggat gtgattattt tcatctctct ggaaccagtg ttacagtact cacagtacct 3000
    gaggtctcgg cagaggatct gtaagagctc catcctccag tggcccaaca atcccaaagc 3060
    agaaaacttg ttttggcaaa gtctgaaaaa tgtggtcttg actgaaaatg attcacggta 3120
30  tgacgatttg tacattgatt ccattaggca atactagtga tgggaagtca cgactctgcc 3180
    atcataaaaa cacacagctt ctcttacaa tgaaccgaat 3220

```

Nucleotide and amino acid sequences of human and murine TLR9 are known. See, for example, GenBank Accession Nos. NM\_017442, AF259262, AB045180, AF245704, AB045181, AF348140, AF314224, NM\_031178; and NP\_059138, AAF 72189, BAB19259, AAF78037, BAB19260, AAK29625, AAK28488, NP\_112455. Human TLR9 is reported to exist in at least two isoforms, one 1032 amino acids long having a sequence provided in SEQ ID NO:34, and the other 1055 amino acids long having a sequence as provided in SEQ ID NO:36. Corresponding nucleotide sequences are provided as SEQ ID NO:35 and SEQ ID NO:37, respectively. The shorter of these two isoforms is believed to be more important. Murine TLR9 is 1032 amino acids long and has a sequence as provided in SEQ ID NO:38. A corresponding nucleotide sequence is provided as SEQ ID NO:39. TLR9 polypeptide includes an extracellular domain having leucine-rich repeat region, a transmembrane domain, and an intracellular domain that includes a TIR domain.

As used herein a "TLR9 polypeptide" refers to a polypeptide including a full-length TLR9 according to one of the sequences above, orthologs, allelic variants, SNPs, variants incorporating conservative amino acid substitutions, TLR9 fusion proteins, and functional

- 42 -

fragments of any of the foregoing. Preferred embodiments include human TLR9 polypeptides having at least 65 percent sequence identity, more preferably at least 80 percent sequence identity, even more preferably with at least 90 percent sequence identity, and most preferably with at least 95 percent sequence identity with the human TLR9 amino acid sequence of SEQ ID NO:34. Preferred embodiments also include murine TLR9 polypeptides having at least 65 percent sequence identity, more preferably at least 80 percent sequence identity, even more preferably with at least 90 percent sequence identity, and most preferably with at least 95 percent sequence identity with the murine TLR9 amino acid sequence of SEQ ID NO:38.

As used herein "TLR9 signaling" refers to an ability of a TLR9 polypeptide to activate the TLR/IL-1R (TIR) signaling pathway, also referred to herein as the TLR signal transduction pathway. Without meaning to be held to any particular theory, it is believed that the TLR/IL-1R signaling pathway involves signaling via the molecules myeloid differentiation marker 88 (MyD88) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), leading to activation of kinases of the I $\kappa$ B kinase complex and the c-jun NH<sub>2</sub>-terminal kinases (e.g., Jnk 1/2). Häcker H et al. (2000) *J Exp Med* 192:595-600. Changes in TLR9 activity can be measured by assays such as those disclosed herein, including expression of genes under control of  $\kappa$ B-sensitive promoters and enhancers. Such naturally occurring genes include the genes encoding IL-1 $\beta$ , IL-6, IL-8, the p40 subunit of interleukin 12 (IL-12 p40), and the costimulatory molecules CD80 and CD86. Other genes can be placed under the control of such regulatory elements (see below) and thus serve to report the level of TLR9 signaling. Additional nucleotide sequence can be added to SEQ ID NO:35 or SEQ ID NO:39, preferably to the 5' or the 3' end of the open reading frame of SEQ ID NO:35, to yield a nucleotide sequence encoding a chimeric polypeptide that includes a detectable or reporter moiety, e.g., FLAG, luciferase (luc), green fluorescent protein (GFP), and others known by those skilled in the art.

#### SEQ ID NO:34 Human TLR9 amino acid (1032)

MGFCRSALHP	LSLLVQAIML	AMTLALGTLF	AFLPCELQPH	GLVNCNWLFL	KSVPHFSMAA	60
PRGNVTSLSL	SSNRIHHLHD	SDFAHLPCLR	HLNLKWNCPP	VGLSPMHFPC	HMTIEPSTFL	120
AVPTLEELNL	SYNNIMTVPA	LPKSLISLSL	SHTNIMLDS	ASLAGLHALR	FLFMDGNCYY	180
KNPCRQALEV	APGALLGLGN	LTHLSLKYN	LTVVPRNLPS	SLEYLLLSYN	RIVKLAPEDL	240
ANLTALRVLD	VGGNCRCDH	APNPCMECP	HFPQLHPDTF	SHLSRLEGLV	LKDSSLWLNL	300
ASWFRGLGNL	RVLDSLENFL	YKCITKTKAF	QGLTQLRKLN	LSFNYQKRVS	FAHLSLAPSF	360
GSLVALKELD	MHGIFFRSLD	ETTLRPLARL	PMLQTLRLQM	NFINQAQLGI	FRAFPGLRYV	420
DLSDNRISGA	SELTATMGEA	DGGEKVWLQP	GDLAPAPVDT	PSEDFRPNC	STLNFTLDLS	480

- 43 -

	RNNLVTVQPE	MFAQLSHLQC	LRLSHNCISQ	AVNGSQFLPL	TGLQVLDLSH	NKLDLYHEHS	540
	FTELPRLAL	DLSYNSQPFQ	MOGVGHNFSP	VAHLRTLRLH	SLAHNNIHSQ	VSQQLCSTSL	600
	RALDFSGNAL	GHMWAEGDLY	LHFFQGLSGL	IWLDLSQNL	HTLLPQTLRN	LPKSLQVLRL	660
	RDNYLAFFKW	WSLHFLPKLE	VLDLAGNQLK	ALTNGSLPAG	TRLRRLDVSC	NSISFVAPGF	720
5	FSKAKELREL	NLSANALKTV	DHSWFGPLAS	ALQILDVSAN	PLHCACGAAP	MDFLLEVQAA	780
	VPGLPSRVKC	GSPGQLQGLS	IFAQDLRLCL	DEALSWDCFA	LSLLAVALGL	GVPMLHHLCC	840
	WDLWYCFHLC	LAWLPWRGRQ	SGRDEDALPY	DAFVVFDKTQ	SAVADWVYNE	LRGQLEECRG	900
	RWALRLCLEE	RDWLPKTLF	ENLWASVYGS	RKTLFVLAHT	DRVSGLLRAS	FLLAQQRLL	960
	DRKDVVVLVI	LSPDGRRSRY	VRLRQRLCRQ	SVLLWPHQPS	GQSRFQAQLG	MALTRDNHFF	1020
10	YNRNFCQGPT	AE					1032

## SEQ ID NO:35 Human TLR9 nucleotide

	ccgctgctgc	ccctgtggga	agggaacctcg	agtgtgaagc	atccttccct	gtagctgctg	60
	tccagctctgc	ccgccagacc	ctctggagaa	gccccctgcc	cccagcatgg	gtttctgccg	120
15	cagcgccctg	caccgcgtgt	ctctcctggg	gcaggccatc	atgctggcca	tgacctggc	180
	cctgggtacc	ttgcctgcct	tcctaccctg	tgagctccag	ccccacggcc	tggtgaactg	240
	caactggctg	ttcctgaagt	ctgtgcccc	cttctccatg	gcagcacccc	gtggcaatgt	300
	caccagcctt	tccttgtcct	ccaaccgcat	ccaccacctc	catgattctg	actttgccc	360
	cctgcccagc	ctgcggcatc	tcaacctcaa	gtggaactgc	ccgccggttg	gcctcagccc	420
20	catgcacttc	ccctgccaca	tgaccatcga	gcccagcacc	ttcttggttg	tgcccacct	480
	ggaagagcta	aacctgagct	acaacaacat	catgactgtg	cctgcgctgc	ccaaatccct	540
	catatccctg	tcctcagcc	ataccaacat	cctgatgcta	gactctgcca	gcctgcctgg	600
	cctgcatgcc	ctgcgcttcc	tattcatgga	cggcaactgt	tattacaaga	acccctgcag	660
	gcaggcactg	gaggtggccc	cgggtgcctt	ccttggtctg	ggcaacctca	cccacctgtc	720
25	actcaagtac	aacaacctca	ctgtgggtgcc	ccgcaacctg	ccttccagcc	tgaggtatct	780
	gctgttgctc	tacaaccgca	tcgtcaaact	ggcgccctgag	gacctggcca	atctgaccgc	840
	cctgcgtgtg	ctcgatgtgg	gcggaaattg	ccgccgctgc	gaccacgctc	ccaacccttg	900
	catggagtg	cctcgctact	tccccagct	acatcccgat	accttcagcc	acctgagccg	960
	tcttgaaagg	ctgggtgtga	aggacagttc	ctctcctgg	ctgaatgcca	gttggttccg	1020
30	tggtgctgga	aacctccgag	tgctggacct	gagtgagaac	ttcctctaca	aatgcatcac	1080
	taaaaccaag	gccttccagg	gcctaacaca	gctgcgcaag	cttaacctgt	ccttcaatta	1140
	ccaaaagagg	gtgtcctttg	cccacctgtc	tctggccctt	tccttcggga	gcctggtcgc	1200
	cctgaaggag	ctggacatgc	acggcatctt	cttccgctca	ctcgatgaga	ccacgctccg	1260
	gccactggcc	cgctgccc	tgctccagac	tctgcgtctg	cagatgaact	tcataacca	1320
35	ggccagctc	ggcatcttca	gggccttccc	tgccctgcgc	tacgtggacc	tgctggacaa	1380
	ccgcatcagc	ggagcttcgg	agctgacagc	caccatgggg	gaggcagatg	gaggggagaa	1440
	ggtctggctg	cagcctgggg	accttgctcc	ggccccagtg	gacactccca	gctctgaaga	1500
	cttcaggccc	aactgcagca	ccctcaactt	caccttggtg	ctgtcacgga	acaacctggt	1560
	gaccgtgcag	ccggagatgt	ttgcccagct	ctcgacacct	cagtgcctgc	gcctgagcca	1620
40	caactgcate	tcgcaggcag	tcaatggctc	ccagttcctg	ccgctgaccg	gtctgcagggt	1680
	gctagacctg	tcccacaata	agctggacct	ctaccacgag	cactcattca	cggagctacc	1740
	acgactggag	gccttgacc	tcagctacaa	cagccagccc	tttggcatgc	agggcgtggg	1800
	ccacaacttc	agcttcgtgg	ctcacctgcg	cacctgcgc	cacctcagcc	tgccccacaa	1860
	caacatccac	agccaagtgt	cccagcagct	ctgcagtacg	tcgctgcggg	ccctggactt	1920
45	cagcggcaat	gcactggggc	atatgtgggc	cgaggagagc	ctctatctgc	acttcttcca	1980
	aggcctgagc	ggtttgatct	ggctggactt	gtcccagaac	cgctgcaca	ccctcctgcc	2040
	ccaaaccttg	cgcaacctcc	ccaagagcct	acaggtgctg	cgctctccgtg	acaattacct	2100
	ggccttcttt	aagtgggtga	gcctccactt	cctgccccaa	ctggaagtcc	tcgacctggc	2160
	aggaaaccag	ctgaaggccc	tgaccaatgg	cagctgcctt	gctggcacc	ggctccggag	2220
50	gctggatgtc	agctgcaaca	gcacagctt	cgtggcccc	ggcttctttt	ccaaggccaa	2280
	ggagctgcga	gagctcaacc	ttagcgccaa	cgccctcaag	acagtggacc	actcctgggt	2340
	tggtggccctg	gcgagtgc	tgcaataact	agatgtaagc	gccaaacctc	tgactgcgc	2400
	ctgtggggcg	gcctttatgg	acttcctgct	ggaggtgcag	gctgccgtgc	ccggtctgcc	2460
	cagccgggtg	aagtgtggca	gtccggggca	gctccagggc	ctcagcatct	ttgcacagga	2520
55	cctgcgcctc	tgctggatg	aggccctctc	ctgggactgt	ttcgccctct	cgctgctggc	2580
	tgtggctctg	ggcctgggtg	tgcccatgct	gcacacctc	tgtggctggg	acctctggta	2640
	ctgcttccac	ctgtgcctgg	cctggcttcc	ctggcgggg	cggcacaaag	ggcgagatga	2700
	ggatgccctg	ccctacgatg	ccttcgtggt	cttcgacaaa	acgcagagcg	cagtggcaga	2760

- 44 -

	ctgggtgtac	aacgagcttc	gggggcagct	ggaggagtgc	cgtgggcgct	gggcactccg	2820
	cctgtgcctg	gaggaacgcg	actggctgcc	tggcaaaacc	ctctttgaga	acctgtgggc	2880
	ctcgtctat	ggcagccgca	agacgctgtt	tgtgctggcc	cacacggacc	gggtcagtg	2940
	tctcttgccg	gccagcttcc	tgctggccca	gcagcgctg	ctggaggacc	gcaaggacgt	3000
5	cgtggtgctg	gtgacccctga	gcctgacgg	ccgccgctcc	cgctacgtgc	ggctgcgcca	3060
	gcgcctctgc	cgccagagtg	tcctcctctg	gccccaccag	cccagtggtc	agcgcagctt	3120
	ctggggccag	ctgggcatgg	ccctgaccag	ggacaaccac	cacttctata	accggaactt	3180
	ctgccaggga	cccacggccg	aatagccgtg	agccggaatc	ctgcacgggtg	ccacctccac	3240
	actcacctca	cctctgc					3258

10

## SEQ ID NO:36 Human TLR9 amino acid (1055)

	MPMKWSGWRW	SWGPATHTAL	PPPQGFCRSA	LHPLSLLVQA	IMLAMTLALG	TLPAFLPCEL	60
	QPHGLVNCNW	LFLKSVPHFS	MAAPRGNVTS	LSLSSNRIHH	LHDSDFAHLP	SLRHLNLKWN	120
	CPPVGLSPMH	FPCHMTIEPS	TFLAVPTLEE	LNLSYNNIMT	VPALPKSLIS	LSLSHTNIML	180
15	LDSASLAGLH	ALRFLFMDGN	CYYKNPCRQA	LEVAPGALLG	LGNLTHLSLK	YNNLTVVPRN	240
	LPSSLEYLLL	SYNRIVKLAP	EDLANLTALR	VLDVGGNCRR	CDHAPNPCME	CPRHFPQLHP	300
	DTFSHLRLE	GLVLKDSSL	WLNASWFRGL	GNLRVLDLSE	NFLYKCITKT	KAFQPLQTLR	360
	KLNLSEFNYQK	RVSFAHLSLA	PSFGSLVALK	ELDMHGIFFR	SLDETTLRPL	ARLPMLQTLR	420
	LQMNFINQAQ	LGIFRAFPGL	RYVDLSNRI	SGASELTATM	GEADGGEKVV	LQPGDLAPAP	480
20	VDTPSSEDFR	PNCSTLNFTL	DLSRNNLVT	QPEMFAQLSH	LQCLRLSHNC	ISQAVNGSQF	540
	LPLTGLQVLD	LSHNKLDLYH	EHSFTLPR	EALDLSYNSQ	PFGMQGVGHN	FSFVAHLRTL	600
	RHLSLAHNNI	HSQVSQQLCS	TSRLALDFSG	NALGHMWAEG	DLYLHFFQGL	SGLIWLDSLQ	660
	NRLHTLLPQT	LRNLPKSLQV	LRLRDNYLAF	FKWWSLHFLP	KLEVLDLAGN	QLKALTNGLS	720
	PAGTRLRRLD	VSCNSISFVA	PGFFSKAKEL	RELNLSANAL	KTVDSHWFPG	LASALQILDV	780
25	SANPLHCACG	AAFMDFLLEV	QAAVPGPSR	VKCGSPGQLQ	GLSIFAQDLR	LCLDEALSWD	840
	CFALSLLAVA	LGLGVPMHH	LCGWDLWYCF	HLCLAWLPWR	GRQSGRDEDA	LPYDAFVVD	900
	KTQSAVADWV	YNELRGQLEE	CRGRWALRLC	LEERDWLPGK	TLFENLWASV	YGSRKTLFVL	960
	AHTDRVSGLL	RASFLLAQQR	LLEDKDVVV	LVILSPDGRR	SRYVRLRQRL	CRQSVLLWPH	1020
	QPSGQRSFWA	QLGMALTRDN	HHFYNRNFCQ	GPTAE			1055

30

## SEQ ID NO:37 Human TLR9 nucleotide

	atgcccattga	agtggagtg	gtggaggtg	agctgggggc	cggccactca	cacagccctc	60
	ccacccccac	aggggttctg	ccgcagcgcc	ctgcacccgc	tgtctctcct	ggtgcaggcc	120
	atcatgctgg	ccatgacct	ggcctgggt	accttgcctg	ccttctctacc	ctgtgagctc	180
35	cagccccacg	gcctggtgaa	ctgcaactgg	ctgttctctga	agtctgtgcc	ccacttctcc	240
	atggcagcac	cccgtggcaa	tgtcaccagc	ctttccttgt	cctccaaccg	catccaccac	300
	ctccatgatt	ctgactttgc	ccacctgccc	agcctgcggc	atctcaacct	caagtggaac	360
	tgcccgccgg	ttggcctcag	ccccatgcac	ttcccctgcc	acatgaccat	cgagcccagc	420
	accttcttgg	ctgtgcccc	cctggaagag	ctaaacctga	gctacaacaa	catcatgact	480
40	gtgcctgcgc	tgcacaaatc	cctcatatcc	ctgtccctca	gccataccaa	catcctgatg	540
	ctagactctg	ccagcctcgc	cggcctgcac	gccctgcgct	tcctattcat	ggacggcaac	600
	tgtattaca	agaacccctg	caggcaggca	ctggaggtgg	ccccgggtgc	cctccttggc	660
	ctgggcaacc	tcacccacct	gtcactcaag	tacaacaacc	tcactgtggt	gccccgcaac	720
	ctgccttcca	gcctggagta	tctgctgttg	tcctacaacc	gcatcgtaa	actggcgctc	780
45	gaggacctgg	ccaatctgac	cgccctgcgt	gtgctcgatg	tgggcggaaa	tgcccgccgc	840
	tgcgaccacg	ctcccaaccc	ctgcatggag	tgcctcgtc	acttcccca	gctacatccc	900
	gataccttca	gccacctgag	ccgtcttgaa	ggcctgggtg	tgaaggacag	ttctctctcc	960
	tggctgaatg	ccagttggtt	ccgtgggctg	ggaaacctcc	gagtgctgga	cctgagtgag	1020
	aacttctct	acaaatgcat	cactaaaacc	aaggccttcc	agggcctaac	acagctgcgc	1080
50	aagcttaacc	tgtccttcaa	ttacaaaag	aggggtgtct	ttgcccacct	gtctctggcc	1140
	ccttctctcg	ggagcctgg	cgccctgaag	gagctggaca	tgcacggcat	cttcttccgc	1200
	tactcgatg	agaccacgct	ccggccactg	gcccgcctgc	ccatgctcca	gactctgcgt	1260
	ctgcagatga	acttcatcaa	ccaggcccag	ctcggcatct	tcagggcctt	ccctggcctg	1320
	cgctacgtgg	acctgtcgga	caaccgcac	agcggagctt	cggagctgac	agccaccatg	1380
55	ggggaggcag	atggagggga	gaaggtctgg	ctgcagcctg	gggaccttgc	tccggcccca	1440
	gtggacactc	ccagctctga	agacttcagg	cccaactgca	gcaccctcaa	cttcaccttg	1500

- 45 -

5	gatctgtcac	ggaacaacct	ggtgaccgtg	cagccggaga	tgtttgcca	gctctcgac	1560
	ctgcagtgcc	tgcgctgag	ccacaactgc	atctcgagg	cagtcaatgg	ctcccagttc	1620
	ctgccgctga	ccggtctgca	ggtgctagac	ctgtcccaca	ataagctgga	cctctaccac	1680
	gagcactcat	tcacggagct	accacgactg	gaggccctgg	acctcagcta	caacagccag	1720
	ccctttggca	tgaggggcgt	gggccacaac	ttcagcttcg	tggctcacct	gcgcaccctg	1800
	cgccacctca	gcctggccca	caacaacatc	cacagccaag	tgtcccagca	gctctgcagt	1860
	acgtcgctgc	gggccctgga	cttcagcggc	aatgcactgg	gccatatgtg	ggccgaggga	1920
	gacctctatc	tgcaacttct	ccaaggcctg	agcggtttga	tctggttgga	cttgtcccag	1980
	aaccgcctgc	acaccctcct	gccccaaacc	ctgcgcaacc	tccccaaagag	cctacagggtg	2040
10	ctgcgctctcc	gtgacaatta	cctggccttc	tttaagtggg	ggagcctcca	cttcctgccc	2100
	aaactggaag	tctcgacct	ggcaggaaac	cagctgaagg	ccctgaccaa	tggcagcctg	2160
	cctgctggca	cccggctccg	gaggctggat	gtcagctgca	acagcatcag	cttcgtggcc	2220
	cccggcttct	tttccaaggc	caaggagctg	cgagagctca	accttagcgc	caacgccctc	2280
	aagacagtgg	accactcctg	gtttggggcc	ctggcgagtg	ccctgcaaat	actagatgta	2340
15	agcgccaacc	ctctgcactg	cgctgtggg	gcggccttta	tggacttct	gctggagggtg	2400
	caggctgccg	tgcccgggtc	ggccagccgg	gtgaagtgtg	gcagtccggg	ccagctccag	2460
	ggcctcagca	tctttgcaca	ggacctgcgc	ctctgcctgg	atgaggccct	ctcctgggac	2520
	tgtttcgccc	tctcgctgtg	ggctgtggct	ctgggcctgg	gtgtgcccac	gctgcacac	2580
	ctctgtggct	gggacctctg	gtactgtctc	cacctgtgcc	tggcctggct	tcctctcgcg	2640
20	ggggcgcaaa	gtgggcgaga	tgaggatgcc	ctgccctacg	atgccttcgt	ggctctcgac	2700
	aaaacgcaga	gcgcagtggc	agactgggtg	tacaacgagc	ttcgggggca	gctggaggag	2760
	tgccgtgggc	gctgggcact	ccgcctgtgc	ctggaggaac	gcgactggct	gcctggcaaa	2820
	accctctttg	agaacctgtg	ggcctcggtc	tatggcagcc	gcaagacgct	gtttgtgctg	2880
	ggccacacgg	accgggtcag	tggtctcttg	cgcgccagct	tcctgctggc	ccagcagcgc	2940
25	ctgctggatg	accgcaagga	cgctgtgggt	ctggtgatcc	tgagccctga	cggccgcgcg	3000
	tcccgcttag	tgcggctgcg	ccagcgcttc	tgccgcagca	gtgtcctcct	ctggccccac	3060
	cagcccagtg	gtcagcgag	cttctggggc	cagctgggca	tggccctgac	cagggacaac	3120
	caccacttct	ataaccgga	cttctgccag	ggacccacgg	ccgaa		3165

## 30 SEQ ID NO:38 Murine TLR9 amino acid

35	MVLRRLTLHP	LSELLVQAAVL	AETLALGTLF	AFLPCELKPH	GLVDCNWLFL	KSVPRFSAAA	60
	SCSNITRLSL	ISNRIHHLHN	SDFVHLSNLR	QLNLKWNCP	TGLSPLHFSC	HMTIEPRTFL	120
	AMRTLEELNL	SYNGITTVPR	LPSSLVNLNL	SHTNIVLDA	NSLAGLYSLR	VLFMDGNCY	180
	KNPCTGAVKV	TPGALLGLSN	LTHLSLKYN	LTKVPRQLPP	SLEYLLVSYN	LIVKLGPEL	240
	ANLTSLRVLD	VGGNCRRCDH	APNPICIECG	KSLHLHPETF	HHLSHLEGLV	LKDSGLTLN	300
	SSWFQGLVNL	SVLDLSENFL	YESINHTNAF	QNLTRLRLKN	LSFNRYKKVS	FARLHLASSF	360
	KNLVSLQELN	MNGIFFRSLN	KYTLRWLADL	PKLHTLHLQM	NFINQAQLSI	FGTFRALRFV	420
	DLSDNRISGP	STLSEATPEE	ADDAEQEELL	SADPHAPPLS	TPASKNFMDR	CKNFKFTMDL	480
	SRNNLVTIKP	EMFVNLSRLQ	CLSLSHNSIA	QAVNGSQFLP	LTNLQVLDLS	HNKLDLYHWK	540
40	SFSELPQLQA	LDLSYNSQPF	SMKGIGHNFS	FVAHLSMLHS	LSLAHNDIHT	RVSSHLNSNS	600
	VRFLDFSGNG	MGRMWDEGGL	YLHFFQGLSG	LLKLDLSQNN	LHILRPQNLD	NLPKSLKLLS	660
	LRDNYLSFFN	WTSLSFLPNL	EVLDLAGNQL	KALTNGTLPN	GTLLQKLDVS	SNSIVSVVPA	720
	FFALAVELKE	VNLSHNLT	VDRSWFGPIV	MNLTVLDVRS	NPLHCACGAA	FVDLLLEVQT	780
	KVPGLANGVK	CGSPGQLQGR	SIFAQDLRLC	LDEVLSWDCF	GLSLLAVAVG	MVVPILHHL	840
45	GWDVWYCFHL	CLAWLPLLAR	SRRSAQALPY	DAFVVFDKAQ	SAVADWVYNE	LRVRLEERRG	900
	RRALRLCLED	RDWLPQTTF	ENLWASIYGS	RKTLFVLAHT	DRVSGLLRTS	FLLAQQRLL	960
	DRKDVVVLVI	LRPDAHRSRY	VRLRQRLCRQ	SVLFWPQPN	QGGFWAQLS	TALTRDNRHF	1020
	YNQNFRCRGPT	AE					1032

## 50 SEQ ID NO:39 Murine TLR9 nucleotide

55	tgtcagaggg	agcctcgagg	gaatcctcca	tctcccaaca	tggttctccg	tcgaaggact	60
	ctgcacccct	gtccctcct	ggtacaggct	gcagtctgg	ctgagactct	ggccctgggt	120
	accctgcctg	ccttctacc	ctgtgagctg	aagcctcatg	gcctgggtgga	ctgcaattgg	180
	ctgttctctga	agtctgtacc	ccgtttctct	gcggcagcat	cctgctccaa	catcacccgc	240
	ctctccttga	tctccaaccg	tatccaccac	ctgcacaact	ccgacttcgt	ccacctgtcc	300
	aacctgcggc	agctgaacct	caagtggaa	tgtccacca	ctggccttag	ccccctgcac	360

- 46 -

	ttctcttgcc	acatgaccat	tgagcccaga	accttcctgg	ctatgcgtac	actggaggag	420
	ctgaacctga	gctataatgg	tatcaccact	gtgccccgac	tgcccagctc	cctgggtgaat	480
	ctgagcctga	gccacaccaa	catcctgggt	ctagatgcta	acagcctcgc	cggcctatac	540
5	agcctgcgcg	ttctcttcat	ggacgggaac	tgctactaca	agaacccctg	cacaggagcg	600
	gtgaagggtga	ccccaggcgc	cctcctgggc	ctgagcaatc	tcacccatct	gtctctgaag	660
	tataacaacc	tcacaaaggt	gccccgcaa	ctgcccccca	gcctggagta	cctcctgggtg	720
	tcctataaacc	tcattgtcaa	gctggggcct	gaagacctgg	ccaatctgac	ctcccttcca	780
	gtacttgatg	tgggtgggaa	ttgccgtcgc	tgcgaccatg	cccccaatcc	ctgtatagaa	840
	tgtggccaaa	agtccttcca	cctgcaccct	gagaccttcc	atcacctgag	ccatctggaa	900
10	ggcctgggtgc	tgaaggacag	ctctctccat	acactgaact	cttcctgggt	ccaaggtctg	960
	gtcaacctct	cgggtgctgga	cctaagcgag	aactttctct	atgaaagcat	caaccacacc	1020
	aatgcctttc	agaacctaac	cgcctgcgc	aagctcaacc	tgtccttcaa	ttaccgcaag	1080
	aaggatctct	ttgcccgcct	ccacctggca	agttccttca	agaacctggg	gtcactgcag	1140
	gagctgaaca	tgaacggcat	cttcttccgc	tcgctcaaca	agtacacgct	cagatggctg	1200
15	gccgatctgc	ccaaactcca	cactctgcac	cttcaaatga	acttcatcaa	ccaggcacag	1260
	ctcagcatct	ttggtacctt	ccgagccctt	cgctttgtgg	acttgtcaga	caatcgcatc	1320
	agtgggcctt	caacgctgtc	agaagccacc	cctgaagagg	cagatgatgc	agagcaggag	1380
	gagctgttgt	ctgcggatcc	tcacccagct	ccactgagca	cccctgcttc	taagaacttc	1440
	atggacaggt	gtaagaactt	caagttcacc	atggacctgt	ctcggaacaa	cctgggtgact	1500
20	atcaagccag	agatgtttgt	caatctctca	cgcctccagt	gtcttagcct	gagccacaac	1560
	tccattgcac	aggctgtcaa	tggtcttcag	ttcctgccgc	tgactaatct	gcaggtgctg	1620
	gacctgtccc	ataacaaact	ggacttgtag	cactggaaat	cgttcagtga	gctaccacag	1680
	ttgcaggccc	tggacctgag	ctacaacagc	cagcccttta	gcatgaaggg	tataggccac	1740
	aatttcagtt	ttgtggccca	tctgtccatg	ctacacagcc	ttagcctggc	acacaatgac	1800
25	attcataccc	gtgtgtcctc	acatctcaac	agcaactcag	tgagggtttct	tgacttcagc	1860
	ggcaacggta	tggggccgcat	gtgggatgag	gggggccttt	atctccattt	cttccaaggc	1920
	ctgagtggcc	tgtgtgaagt	ggacctgtct	caaaataacc	tgcataatct	ccggccccag	1980
	aaccttgaca	acctcccca	gagcctgaag	ctgctgagcc	tccgagacaa	ctacctatct	2040
	ttctttaact	ggaccagtct	gtccttctctg	cccaacctgg	aagtcctaga	cctggcaggc	2100
30	aaccagctaa	aggccctgac	caatggcacc	ctgcctaattg	gcacctcctc	ccagaaactg	2160
	gatgtcagca	gcaacagtat	cgtctctgtg	gtcccagcct	tcttcgctct	ggcggtcgag	2220
	ctgaaagagg	tcaacctcag	ccacaacatt	ctcaagacgg	tggatcgctc	ctgggttggg	2280
	cccattgtga	tgaacctgac	agttctagac	gtgagaagca	accctctgca	ctgtgcttgt	2340
	ggggcagcct	tcgtagactt	actgttggag	gtgcagacca	aggtgcctgg	cctggctaata	2400
35	gggtggaagt	gtggcagccc	cggccagctg	cagggccgta	gcatcttcgc	acaggacctg	2460
	cggctgtgcc	tggatgaggt	cctctcttgg	gactgctttg	gcctttcact	cttggctgtg	2520
	gccgtgggca	tgggtgtgcc	tatactgcac	catctctgcg	gctgggacgt	ctgggtactgt	2580
	tttcatctgt	gcctggcatg	gctacctttg	ctggcccgcg	gccgacgcag	cgcccaagct	2640
	ctcccctatg	atgccttcgt	gggtgttcgat	aaggcacaga	gcgcagttgc	ggactgggtg	2700
40	tataacgagc	tgcgggtgcg	gctggaggag	cggcgcggtc	gccgagccct	acgcttgtgt	2760
	ctggaggacc	gagattggct	gcctggccag	acgctcttcg	agaacctctg	ggcttccatc	2820
	tatgggagcc	gcaagactct	atgtgtgctg	gcccacacgg	accgcgtcag	tggcctcctg	2880
	cgcaccagct	tcctgctggc	tcagcagcgc	ctggttgaag	accgcaagga	cgtgggtggg	2940
	ttggtgatcc	tgcgtccgga	tgccaccgcg	tcccgcctatg	tgcgactgcg	ccagcgtctc	3000
45	tgccgccaga	gtgtgctctt	ctggccccag	cagcccaacg	ggcagggggg	cttctggggc	3060
	cagctgagta	cagccctgac	tagggacaac	cgcacttctt	ataaccagaa	cttctgcccg	3120
	ggacctacag	cagaatagct	cagagcaaca	gctggaaaca	gctgcatctt	catgcctggg	3180
	tcccagattg	ctctgcctgc					3200

50 Ribonucleoside vanadyl complexes (i.e., mixtures of adenine, cytosine, guanosine, and uracil ribonucleoside vanadyl complexes), are well known by those of skill in the art as RNase inhibitors. Berger SL et al. (1979) *Biochemistry* 18:5143; Puskas RS et al. (1982) *Biochemistry* 21:4602. Ribonucleoside vanadyl complexes are commercially available from suppliers including Sigma-Aldrich, Inc.

- 47 -

In one embodiment, the immunostimulatory G,U-containing RNA oligomer of the invention does not contain a CpG dinucleotide and is not a CpG immunostimulatory nucleic acid. In some embodiments, a CpG immunostimulatory nucleic acid is used in the methods of the invention.

5 A CpG immunostimulatory nucleic acid is a nucleic acid which contains a CG dinucleotide, the C residue of which is unmethylated. CpG immunostimulatory nucleic acids are known to stimulate Th1-type immune responses. CpG sequences, while relatively rare in human DNA are commonly found in the DNA of infectious organisms such as bacteria. The human immune system has apparently evolved to recognize CpG sequences as an early  
10 warning sign of infection and to initiate an immediate and powerful immune response against invading pathogens without causing adverse reactions frequently seen with other immune stimulatory agents. Thus CpG containing nucleic acids, relying on this innate immune defense mechanism can utilize a unique and natural pathway for immune therapy. The effects of CpG nucleic acids on immune modulation have been described extensively in U.S.  
15 patents such as US 6,194,388 B1, US 6,207,646 B1, US 6,239,116 B1 and US 6,218,371 B1, and published patent applications, such as PCT/US98/03678, PCT/US98/10408, PCT/US98/04703, and PCT/US99/09863. The entire contents of each of these patents and patent applications is hereby incorporated by reference.

A CpG nucleic acid is a nucleic acid which includes at least one unmethylated CpG  
20 dinucleotide. A nucleic acid containing at least one unmethylated CpG dinucleotide is a nucleic acid molecule which contains an unmethylated cytosine in a cytosine-guanine dinucleotide sequence (i.e., "CpG DNA" or DNA containing a 5' cytosine followed by 3' guanosine and linked by a phosphate bond) and activates the immune system. The CpG nucleic acids can be double-stranded or single-stranded. Generally, double-stranded  
25 molecules are more stable *in vivo*, while single-stranded molecules have increased immune activity. Thus in some aspects of the invention it is preferred that the nucleic acid be single stranded and in other aspects it is preferred that the nucleic acid be double stranded. In certain embodiments, while the nucleic acid is single stranded, it is capable of forming secondary and tertiary structures (e.g., by folding back on itself, or by hybridizing with itself  
30 either throughout its entirety or at select segments along its length). Accordingly, while the primary structure of such a nucleic acid may be single stranded, its higher order structures may be double or triple stranded. The terms CpG nucleic acid or CpG oligonucleotide as

- 48 -

used herein refer to an immunostimulatory CpG nucleic acid unless otherwise indicated. The entire immunostimulatory nucleic acid can be unmethylated or portions may be unmethylated but at least the C of the 5' CG 3' must be unmethylated.

In one aspect the invention provides a method of activating an immune cell. The method involves contacting an immune cell with an immunostimulatory composition of the invention, described above, in an effective amount to induce activation of the immune cell. As used herein, an "immune cell" is cell that belongs to the immune system. Immune cells participate in the regulation and execution of inflammatory and immune responses. They include, without limitation, B lymphocytes (B cells), T lymphocytes (T cells), natural killer (NK) cells, dendritic cells, other tissue-specific antigen-presenting cells (e.g., Langerhans cells), macrophages, monocytes, granulocytes (neutrophils, eosinophils, basophils), and mast cells. Splenocytes, thymocytes, and peripheral blood mononuclear cells (PBMCs) include immune cells. Immune cells can be isolated from the blood, spleen, marrow, lymph nodes, thymus, and other tissues using methods well known to those of skill in the art. Immune cells can also include certain cell lines as well as primary cultures maintained in vitro or ex vivo.

In one embodiment the activation of the immune cell involves secretion of a cytokine by the immune cell. In one embodiment the activation of the immune cell involves secretion of a chemokine by the immune cell. In one embodiment the activation of the immune cell involves expression of a costimulatory/accessory molecule by the immune cell. In one embodiment the costimulatory/accessory molecule is selected from the group consisting of intercellular adhesion molecules (ICAMs, e.g., CD54), leukocyte function-associated antigens (LFAs, e.g., CD58), B7s (CD80, CD86), and CD40.

"Activation of an immune cell" shall refer to a transition of an immune cell from a resting or quiescent state to a state of heightened metabolic activity and phenotype associated with immune cell function. Such immune cell function can include, for example, secretion of soluble products such as immunoglobulins, cytokines, and chemokines; cell surface expression of costimulatory/accessory molecules and MHC antigens; immune cell migration; phagocytosis and cytotoxic activity toward target cells; and immune cell maturation. In some instances immune activation can refer to Th1 immune activation; in other instances immune activation can refer to Th2 immune activation.

"Th1 immune activation" as used herein refers to the activation of immune cells to express Th1-like secreted products, including certain cytokines, chemokines, and subclasses

- 49 -

of immunoglobulin; and activation of certain immune cells. Th1-like secreted products include, for example, the cytokines IFN- $\gamma$ , IL-2, IL-12, IL-18, TNF- $\alpha$ , and the chemokine IP-10 (CXCL10). In the mouse, Th1 immune activation stimulates secretion of IgG2a. Th1 immune activation also may include activation of NK cells and dendritic cells, i.e., cells involved in cellular immunity. Th1 immune activation is believed to counter-regulate Th2 immune activation.

“Th2 immune activation” as used herein refers to the activation of immune cells to express Th2-like secreted products, including certain cytokines and subclasses of immunoglobulin. Th2-like secreted products include, for example, the cytokines IL-4 and IL-10. In the mouse, Th2 immune activation stimulates secretion of IgG1 and IgE. Th2 immune activation is believed to counter-regulate Th1 immune activation.

In another aspect, the invention provides a method of inducing an immune response in a subject. The method entails administering to a subject a composition of the invention in an effective amount to induce an immune response in the subject. Thus the compositions of the invention may be used to treat a subject in need of immune activation. A subject in need of immune activation may include a subject in need of Th1-like immune activation.

The compositions and methods of the invention can be used, alone or in conjunction with other agents, to treat a subject in need of Th1-like immune activation. A “subject in need of Th1-like immune activation” is a subject that has or is at risk of developing a disease, disorder, or condition that would benefit from an immune response skewed toward Th1. Such a subject may have or be at risk of having a Th2-mediated disorder that is susceptible to Th1-mediated cross-regulation or suppression. Such disorders include, for example, certain organ-specific autoimmune diseases. Alternatively, such a subject may have or be at risk of having a Th1-deficient state. Such disorders include, for example, tumors, infections with intracellular pathogens, and AIDS.

As used herein, “G,U-rich RNA” shall mean RNA at least 5 nucleotides long that by base composition is at least 60 percent, more preferably at least 80 percent, and most preferably at least 90 percent guanine (G) and uracil (U). Such base composition is measured over the full length of the RNA if it is no more than 10 bases long, and over a stretch of at least 10 contiguous bases if the RNA is more than 10 bases long.

As used herein, “G-rich RNA” shall mean RNA that by base composition is at least 70 percent, more preferably at least 80 percent, even more preferably at least 90 percent, and

- 50 -

most preferably at least 95 percent guanine (G). Such base composition is measured over the full length of the RNA if it is no more than 10 bases long, and over a stretch of at least 10 contiguous bases if the RNA is more than 10 bases long.

In some embodiments the compositions of the present invention include a DNA:RNA conjugate. A DNA:RNA conjugate shall mean a molecule or complex that includes at least one deoxyribonucleoside linked to at least one ribonucleoside. The deoxyribonucleoside and ribonucleoside components may be linked by base pair interaction. Alternatively, the deoxyribonucleoside and ribonucleoside components may be linked by covalent linkage between the sugar moieties of the at least one deoxyribonucleoside and the at least one ribonucleoside. The covalent linkage between the sugar moieties may be direct or indirect, for example through a linker. Base pair interactions typically are, but are not limited to, non-covalent Watson-Crick type base pair interactions. Other base pair interactions, including non-covalent (e.g., Hoogsteen base pairing) and covalent interactions are contemplated by the invention. Base pair interactions also typically will involve duplex formation involving two strands, but higher order interactions are also contemplated by the invention.

A DNA:RNA conjugate involving a covalent linkage between the sugar moieties of the at least one deoxyribonucleoside and the at least one ribonucleoside is referred to herein as having a chimeric DNA:RNA backbone. The DNA:RNA conjugate having a chimeric DNA:RNA backbone will have primary structure defined by its base sequence, and it may further have a secondary or higher order structure. A secondary or higher order structure will include at least one intramolecular base pair interaction, e.g., a stem-loop structure, or intermolecular base pair interaction.

Heteroduplex base pairing shall refer to intramolecular or intermolecular base pair interaction between DNA and RNA. For example, heteroduplex base pairing may occur between individual complementary single-stranded DNA and RNA molecules. Alternatively, as in the case of suitable DNA:RNA chimeric backbone nucleic acid molecules, heteroduplex base pairing may occur between complementary DNA and RNA regions within the same molecule.

In some embodiments the compositions of the present invention include a chimeric DNA:RNA backbone having a cleavage site between the DNA and RNA. A cleavage site refers to a structural element along the chimeric backbone that is susceptible to cleavage by any suitable means. The cleavage site may be a phosphodiester bond that is relatively

- 51 -

susceptible to cleavage by endonuclease. In this instance the DNA and RNA each may include internucleotide linkages that are stabilized, such that the chimeric backbone is most susceptible to endonuclease cleavage at the phosphodiester junction between the stabilized DNA and the stabilized RNA. The cleavage site may be designed so that it is susceptible to  
5 cleavage under certain pH conditions, e.g., relatively more stable at higher pH than at lower pH, or vice versa. Such pH sensitivity may be accomplished, for example, by preparation of the chimeric DNA:RNA composition in liposomes. The cleavage site may involve a disulfide linkage. Such disulfide linkage may be relatively more stable under oxidizing conditions than under reducing conditions, e.g., the latter conditions present within an  
10 endosome. The cleavage site may also involve a linker that is susceptible to cleavage by an enzyme, pH, redox condition, or the like. In some embodiments the composition may include more than one cleavage site.

Conjugates of the invention permit selection of fixed molar ratios of the components of the conjugates. In the case of DNA:RNA conjugates it may be advantageous or  
15 convenient to have a 1:1 ratio of DNA and RNA. Conjugates that are heteroduplex DNA:RNA will commonly have a 1:1 ratio of DNA and RNA. Conjugates that have a chimeric DNA:RNA backbone may also commonly have a 1:1 ratio of DNA and RNA. Conjugates having other DNA:RNA ratios are contemplated by the invention, including, but not limited to, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1, and so on. The conjugation may stabilize one or  
20 more components in comparison to the stability of the same component or components alone. Conjugation may also facilitate delivery of the components into cells at the selected ratio.

Cleavage sites may serve any of several purposes useful in the present invention. Once delivered to a cell of interest, the components joined via the cleavage site (or sites) may be liberated to become independently or optimally active within the cell or in the vicinity of  
25 the cell. In some embodiments the cleavage sites may be important to pharmacokinetics of at least one of the components of the conjugate. For instance, the cleavage sites may be designed and selected to confer an extended time release of one of the components.

The invention generally provides efficient methods of identifying immunostimulatory compounds and the compounds and agents so identified. Generally, the screening methods  
30 involve assaying for compounds which inhibit or enhance signaling through a particular TLR. The methods employ a TLR, a suitable reference ligand for the TLR, and a candidate immunostimulatory compound. The selected TLR is contacted with a suitable reference

- 52 -

compound (TLR ligand) and a TLR-mediated reference signal is measured. The selected TLR is also contacted with a candidate immunostimulatory compound and a TLR-mediated test signal is measured. The test signal and the reference signal are then compared. A favorable candidate immunostimulatory compound may subsequently be used as a reference compound in the assay. Such methods are adaptable to automated, high throughput screening of candidate compounds. Examples of such high throughput screening methods are described in U.S. Pat. Nos. 6,103,479; 6,051,380; 6,051,373; 5,998,152; 5,876,946; 5,708,158; 5,443,791; 5,429,921; and 5,143,854.

The assay mixture comprises a candidate immunostimulatory compound. Typically, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a different response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration of agent or at a concentration of agent below the limits of assay detection. Candidate immunostimulatory compounds encompass numerous chemical classes, although typically they are organic compounds. Preferably, the candidate immunostimulatory compounds are small organic compounds, i.e., those having a molecular weight of more than 50 yet less than about 2500. Polymeric candidate immunostimulatory compounds can have higher molecular weights, e.g., oligonucleotides in the range of about 2500 to about 12,500. Candidate immunostimulatory compounds comprise functional chemical groups necessary for structural interactions with polypeptides, and may include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups and more preferably at least three of the functional chemical groups. The candidate immunostimulatory compounds can comprise cyclic carbon or heterocyclic structure and/or aromatic or polyaromatic structures substituted with one or more of the above-identified functional groups. Candidate immunostimulatory compounds also can be biomolecules such as nucleic acids, peptides, saccharides, fatty acids, sterols, isoprenoids, purines, pyrimidines, derivatives or structural analogs of the above, or combinations thereof and the like. Where the candidate immunostimulatory compound is a nucleic acid, the candidate immunostimulatory compound typically is a DNA or RNA molecule, although modified nucleic acids having non-natural bonds or subunits are also contemplated.

Candidate immunostimulatory compounds are obtained from a wide variety of sources, including libraries of natural, synthetic, or semisynthetic compounds, or any

- 53 -

combination thereof. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides, synthetic organic combinatorial libraries, phage display libraries of random peptides, and the like. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural and synthetically produced libraries and compounds can be readily modified through conventional chemical, physical, and biochemical means. Further, known pharmacological agents may be subjected to directed or random chemical modifications such as acylation, alkylation, esterification, amidification, etc., to produce structural analogs of the candidate immunostimulatory compounds.

Therefore, a source of candidate immunostimulatory compounds are libraries of molecules based on known TLR ligands, e.g., CpG oligonucleotides known to interact with TLR9, in which the structure of the ligand is changed at one or more positions of the molecule to contain more or fewer chemical moieties or different chemical moieties. The structural changes made to the molecules in creating the libraries of analog inhibitors can be directed, random, or a combination of both directed and random substitutions and/or additions. One of ordinary skill in the art in the preparation of combinatorial libraries can readily prepare such libraries based on existing TLR9 ligands.

A variety of other reagents also can be included in the mixture. These include reagents such as salts, buffers, neutral proteins (e.g., albumin), detergents, etc. which may be used to facilitate optimal protein-protein and/or protein-nucleic acid binding. Such a reagent may also reduce non-specific or background interactions of the reaction components. Other reagents that improve the efficiency of the assay such as protease inhibitors, nuclease inhibitors, antimicrobial agents, and the like may also be used.

The order of addition of components, incubation temperature, time of incubation, and other parameters of the assay may be readily determined. Such experimentation merely involves optimization of the assay parameters, not the fundamental composition of the assay. Incubation temperatures typically are between 4°C and 40°C. Incubation times preferably are minimized to facilitate rapid, high throughput screening, and typically are between 1 minute and 10 hours.

After incubation, the level of TLR signaling is detected by any convenient method available to the user. For cell-free binding type assays, a separation step is often used to

- 54 -

separate bound from unbound components. The separation step may be accomplished in a variety of ways. For example, separation can be accomplished in solution, or, conveniently, at least one of the components is immobilized on a solid substrate, from which the unbound components may be easily separated. The solid substrate can be made of a wide variety of materials and in a wide variety of shapes, e.g., microtiter plate, microbead, dipstick, resin particle, etc. The substrate preferably is chosen to maximize signal-to-noise ratios, primarily to minimize background binding, as well as for ease of separation and cost.

Separation may be effected for example, by removing a bead or dipstick from a reservoir, emptying or diluting a reservoir such as a microtiter plate well, rinsing a bead, particle, chromatographic column or filter with a wash solution or solvent. The separation step preferably includes multiple rinses or washes. For example, when the solid substrate is a microtiter plate, the wells may be washed several times with a washing solution, which typically includes those components of the incubation mixture that do not participate in specific bindings such as salts, buffer, detergent, non-specific protein, etc. Where the solid substrate is a magnetic bead, the beads may be washed one or more times with a washing solution and isolated using a magnet.

Detection may be effected in any convenient way for cell-based assays such as measurement of an induced polypeptide within, on the surface of, or secreted by the cell. Examples of detection methods useful in cell-based assays include fluorescence-activated cell sorting (FACS) analysis, bioluminescence, fluorescence, enzyme-linked immunosorbent assay (ELISA), reverse transcriptase-polymerase chain reaction (RT-PCR), and the like. Examples of detection methods useful in cell-free assays include bioluminescence, fluorescence, enzyme-linked immunosorbent assay (ELISA), reverse transcriptase-polymerase chain reaction (RT-PCR), and the like.

A subject shall mean a human or animal including but not limited to a dog, cat, horse, cow, pig, sheep, goat, chicken, rodent, e.g., rats and mice, primate, e.g., monkey, and fish or aquaculture species such as fin fish (e.g., salmon) and shellfish (e.g., shrimp and scallops). Subjects suitable for therapeutic or prophylactic methods include vertebrate and invertebrate species. Subjects can be house pets (e.g., dogs, cats, fish, etc.), agricultural stock animals (e.g., cows, horses, pigs, chickens, etc.), laboratory animals (e.g., mice, rats, rabbits, etc.), zoo animals (e.g., lions, giraffes, etc.), but are not so limited. Although many of the embodiments

- 55 -

described herein relate to human disorders, the invention is also useful for treating other nonhuman vertebrates.

As used herein, the term "treat", when used with respect to one of the disorders described herein, refers both to a prophylactic treatment which decreases the likelihood that a  
5 subject will develop the disorder as well as to treatment of an established disorder, e.g., to reduce or eliminate the disorder or symptoms of the disorder, or to prevent the disorder or symptoms of the disorder from becoming worse.

A subject that has a disorder refers to a subject that has an objectively measureable manifestation of the disorder. Thus for example a subject that has a cancer is a subject that  
10 has detectable cancerous cells. A subject that has an infection is a subject that has been exposed to an infectious organism and has acute or chronic detectable levels of the organism in the body. The infection may be latent (dormant) or active.

A subject at risk of having a disorder is defined as a subject that has a higher than normal risk of developing the disorder. The normal risk is generally the risk of a population  
15 of normal individuals that do not have the disorder and that are not identifiably predisposed, e.g., either genetically or environmentally, to developing the disorder. Thus a subject at risk of having a disorder may include, without limitation, a subject that is genetically predisposed to developing the disorder, as well as a subject that is or will be exposed to an environmental agent known or believed to cause the disorder. Environmental agents specifically include,  
20 but are not limited to, infectious agents such as viruses, bacteria, fungi, and parasites. Other environmental agents may include, for example, tobacco smoke, certain organic chemicals, asbestos, and the like.

The term "effective amount" of a nucleic acid or other therapeutic agent refers to the amount necessary or sufficient to realize a desired biologic effect. In general, an effective  
25 amount is that amount necessary to cause activation of the immune system, resulting potentially in the development of an antigen-specific immune response. In some embodiments, the nucleic acid or other therapeutic agent are administered in an effective amount to stimulate or induce a Th1 immune response or a general immune response. An effective amount to stimulate a Th1 immune response may be defined as that amount which  
30 stimulates the production of one or more Th1-type cytokines, such as IL-2, IL-12, TNF- $\alpha$ , and IFN- $\gamma$ , and/or production of one or more Th1-type antibodies.

- 56 -

In yet another aspect the invention provides a method of inducing an immune response in a subject. The method according to this aspect of the invention involves administering to a subject an antigen, and administering to the subject an immunostimulatory composition of the invention in an effective amount to induce an immune response to the antigen. It is to be noted that the antigen may be administered before, after, or concurrently with the immunostimulatory composition of the invention. In addition, both the antigen and the immunostimulatory compound can be administered to the subject more than once.

The invention further provides, in yet another aspect, a method of inducing an immune response in a subject. The method according to this aspect of the invention involves isolating dendritic cells of a subject, contacting the dendritic cells *ex vivo* with an immunostimulatory composition of the invention, contacting the dendritic cells *ex vivo* with an antigen, and administering the contacted dendritic cells to the subject.

The term "antigen" refers to a molecule capable of provoking an immune response. The term antigen broadly includes any type of molecule that is recognized by a host system as being foreign. Antigens include but are not limited to microbial antigens, cancer antigens, and allergens. Antigens include, but are not limited to, cells, cell extracts, proteins, polypeptides, peptides, polysaccharides, polysaccharide conjugates, peptide and non-peptide mimics of polysaccharides and other molecules, small molecules, lipids, glycolipids, and carbohydrates. Many antigens are protein or polypeptide in nature, as proteins and polypeptides are generally more antigenic than carbohydrates or fats.

The antigen may be an antigen that is encoded by a nucleic acid vector or it may not be encoded in a nucleic acid vector. In the former case the nucleic acid vector is administered to the subject and the antigen is expressed *in vivo*. In the latter case the antigen may be administered directly to the subject. An antigen not encoded in a nucleic acid vector as used herein refers to any type of antigen that is not a nucleic acid. For instance, in some aspects of the invention the antigen not encoded in a nucleic acid vector is a peptide or a polypeptide. Minor modifications of the primary amino acid sequences of peptide or polypeptide antigens may also result in a polypeptide which has substantially equivalent antigenic activity as compared to the unmodified counterpart polypeptide. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as antigenicity still exists. The peptide or polypeptide may be, for example, virally derived.

- 57 -

The antigens useful in the invention may be any length, ranging from small peptide fragments of a full length protein or polypeptide to the full length form. For example, the antigen may be less than 5, less than 8, less than 10, less than 15, less than 20, less than 30, less than 50, less than 70, less than 100, or more amino acid residues in length, provided it stimulates a specific immune response.

The nucleic acid encoding the antigen is operatively linked to a gene expression sequence which directs the expression of the antigen nucleic acid within a eukaryotic cell. The gene expression sequence is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the antigen nucleic acid to which it is operatively linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase,  $\beta$ -actin promoter and other constitutive promoters.

Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the cytomegalovirus (CMV), simian virus (e.g., SV40), papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, the long terminal repeats (LTR) of Moloney leukemia virus and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

In general, the gene expression sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined antigen nucleic acid. The gene expression sequences optionally include enhancer sequences or upstream activator sequences as desired.

- 58 -

The antigen nucleic acid is operatively linked to the gene expression sequence. As used herein, the antigen nucleic acid sequence and the gene expression sequence are said to be operably linked when they are covalently linked in such a way as to place the expression or transcription and/or translation of the antigen coding sequence under the influence or control of the gene expression sequence. Two DNA sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the antigen sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the antigen sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to an antigen nucleic acid sequence if the gene expression sequence were capable of effecting transcription of that antigen nucleic acid sequence such that the resulting transcript is translated into the desired protein or polypeptide.

The antigen nucleic acid of the invention may be delivered to the immune system alone or in association with a vector. In its broadest sense, a vector is any vehicle capable of facilitating the transfer of the antigen nucleic acid to the cells of the immune system so that the antigen can be expressed and presented on the surface of the immune cell. The vector generally transports the nucleic acid to the immune cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. The vector optionally includes the above-described gene expression sequence to enhance expression of the antigen nucleic acid in immune cells. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the antigen nucleic acid sequences. Viral vectors are a preferred type of vector and include, but are not limited to, nucleic acid sequences from the following viruses: retrovirus, such as Moloney murine leukemia virus, Harvey murine sarcoma virus, murine mammary tumor virus, and Rous sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known in the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses

- 59 -

include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression  
5 vectors have general utility for the high-efficiency transduction of genes *in vivo*. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral  
10 particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M., Gene Transfer and Expression, A Laboratory Manual, W.H. Freeman Co., New York (1990) and Murray, E.J. Methods in Molecular Biology, vol. 7, Humana Press, Inc., Clifton, New Jersey (1991).

A preferred virus for certain applications is the adeno-associated virus, a  
15 double-stranded DNA virus. The adeno-associated virus can be engineered to be replication-deficient and is capable of infecting a wide range of cell types and species. It further has advantages, such as heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hemopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, wild-type adeno-associated virus  
20 manifest some preference for integration sites into human cellular DNA, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable  
25 event. The adeno-associated virus can also function in an extrachromosomal fashion. Recombinant adeno-associated viruses that lack the replicase protein apparently lack this integration sequence specificity.

Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well-known to those of skill in the art. See, e.g., Sambrook et al.,  
30 Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been found to be particularly advantageous for delivering genes to cells *in vivo* because of their inability to replicate within

- 60 -

and integrate into a host genome. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUC19, pRc/CMV, SV40, and pBlueScript. Other plasmids are well-known to those of ordinary skill in the art.

5 Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA.

It has recently been discovered that gene-carrying plasmids can be delivered to the immune system using bacteria. Modified forms of bacteria such as *Salmonella* can be transfected with the plasmid and used as delivery vehicles. The bacterial delivery vehicles  
10 can be administered to a host subject orally or by other administration means. The bacteria deliver the plasmid to immune cells, e.g., B cells, dendritic cells, likely by passing through the gut barrier. High levels of immune protection have been established using this methodology. Such methods of delivery are useful for the aspects of the invention utilizing systemic delivery of antigen, nucleic acids, and/or other therapeutic agent.

15 In some aspects of the invention, the nucleic acids are administered along with therapeutic agents such as disorder-specific medicaments. As used herein, a disorder-specific medicament is a therapy or agent that is used predominately in the treatment or prevention of a disorder.

In one aspect, the combination of nucleic acid and disorder-specific medicaments  
20 allows for the administration of higher doses of disorder-specific medicaments without as many side effects as are ordinarily experienced at those high doses. In another aspect, the combination of nucleic acid and disorder-specific medicaments allows for the administration of lower, sub-therapeutic doses of either compound, but with higher efficacy than would otherwise be achieved using such low doses. As one example, by administering a  
25 combination of an immunostimulatory nucleic acid and a medicament, it is possible to achieve an effective response even though the medicament is administered at a dose which alone would not provide a therapeutic benefit (i.e., a sub-therapeutic dose). As another example, the combined administration achieves a response even though the nucleic acid is administered at a dose which alone would not provide a therapeutic benefit.

30 The nucleic acids and/or other therapeutic agents can also be administered on fixed schedules or in different temporal relationships to one another. The various combinations have many advantages over the prior art methods of modulating immune responses or

- 61 -

preventing or treating disorders, particularly with regard to decreased non-specific toxicity to normal tissues.

Cancer is a disease which involves the uncontrolled growth (i.e., division) of cells. Some of the known mechanisms which contribute to the uncontrolled proliferation of cancer cells include growth factor independence, failure to detect genomic mutation, and  
5 inappropriate cell signaling. The ability of cancer cells to ignore normal growth controls may result in an increased rate of proliferation. Although the causes of cancer have not been firmly established, there are some factors known to contribute, or at least predispose a subject, to cancer. Such factors include particular genetic mutations (e.g., BRCA gene  
10 mutation for breast cancer, APC for colon cancer), exposure to suspected cancer-causing agents, or carcinogens (e.g., asbestos, UV radiation) and familial disposition for particular cancers such as breast cancer.

The cancer may be a malignant or non-malignant cancer. Cancers or tumors include but are not limited to biliary tract cancer; brain cancer; breast cancer; cervical cancer;  
15 choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer; gastric cancer; intraepithelial neoplasms; lymphomas; liver cancer; lung cancer (e.g., small cell and non-small cell); melanoma; neuroblastomas; oral cancer; ovarian cancer; pancreas cancer; prostate cancer; rectal cancer; sarcomas; skin cancer; testicular cancer; thyroid cancer; and renal cancer, as well as other carcinomas and sarcomas. In one embodiment the cancer is  
20 hairy cell leukemia, chronic myelogenous leukemia, cutaneous T-cell leukemia, multiple myeloma, follicular lymphoma, malignant melanoma, squamous cell carcinoma, renal cell carcinoma, prostate carcinoma, bladder cell carcinoma, or colon carcinoma.

A "subject having a cancer" is a subject that has detectable cancerous cells.

A "subject at risk of developing a cancer" is one who has a higher than normal  
25 probability of developing cancer. These subjects include, for instance, subjects having a genetic abnormality that has been demonstrated to be associated with a higher likelihood of developing a cancer, subjects having a familial disposition to cancer, subjects exposed to cancer-causing agents (i.e., carcinogens) such as tobacco, asbestos, or other chemical toxins, and subjects previously treated for cancer and in apparent remission.

30 A "cancer antigen" as used herein is a compound, such as a peptide or protein, associated with a tumor or cancer cell surface and which is capable of provoking an immune response when expressed on the surface of an antigen presenting cell in the context of an

- 62 -

MHC molecule. Cancer antigens can be prepared from cancer cells either by preparing crude extracts of cancer cells, for example, as described in Cohen PA et al. (1994) *Cancer Res* 54:1055-8, by partially purifying the antigens, by recombinant technology, or by de novo synthesis of known antigens. Cancer antigens include but are not limited to antigens that are recombina<sup>5</sup> ntly expressed, an immunogenic portion of, or a whole tumor or cancer. Such antigens can be isolated or prepared recombinantly or by any other means known in the art.

The terms "cancer antigen" and "tumor antigen" are used interchangeably and refer to antigens which are differentially expressed by cancer cells and can thereby be exploited in order to target cancer cells. Cancer antigens are antigens which can potentially stimulate<sup>10</sup> apparently tumor-specific immune responses. Some of these antigens are encoded, although not necessarily expressed, by normal cells. These antigens can be characterized as those which are normally silent (i.e., not expressed) in normal cells, those that are expressed only at certain stages of differentiation and those that are temporally expressed such as embryonic and fetal antigens. Other cancer antigens are encoded by mutant cellular genes, such as<sup>15</sup> oncogenes (e.g., activated *ras* oncogene), suppressor genes (e.g., mutant p53), fusion proteins resulting from internal deletions or chromosomal translocations. Still other cancer antigens can be encoded by viral genes such as those carried on RNA and DNA tumor viruses.

Examples of tumor antigens include MAGE, MART-1/Melan-A, gp100, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (ADAbp), cyclophilin b, Colorectal<sup>20</sup> associated antigen (CRC)--C017-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA), T-cell receptor/CD3-zeta chain, MAGE-family of tumor antigens (e.g., MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8,<sup>25</sup> MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5), GAGE-family of tumor antigens (e.g., GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1,  $\alpha$ -fetoprotein, E-cadherin,  $\alpha$ -catenin,  $\beta$ -catenin and  $\gamma$ -catenin, p120ctn, gp100<sup>Pmel117</sup>, PRAME, NY-ESO-1,<sup>30</sup> cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotypic, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papilloma virus proteins,

Smad family of tumor antigens, Imp-1, P1A, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 and CT-7, and c-erbB-2.

Cancers or tumors and tumor antigens associated with such tumors (but not exclusively), include acute lymphoblastic leukemia (etv6; aml1; cyclophilin b), B cell lymphoma (Ig-idiotypic), glioma (E-cadherin;  $\alpha$ -catenin;  $\beta$ -catenin;  $\gamma$ -catenin; p120ctn), bladder cancer (p21ras), biliary cancer (p21ras), breast cancer (MUC family; HER2/neu; c-erbB-2), cervical carcinoma (p53; p21ras), colon carcinoma (p21ras; HER2/neu; c-erbB-2; MUC family), colorectal cancer (Colorectal associated antigen (CRC)--C017-1A/GA733; APC), choriocarcinoma (CEA), epithelial cell cancer (cyclophilin b), gastric cancer (HER2/neu; c-erbB-2; ga733 glycoprotein), hepatocellular cancer ( $\alpha$ -fetoprotein), Hodgkins lymphoma (Imp-1; EBNA-1), lung cancer (CEA; MAGE-3; NY-ESO-1), lymphoid cell-derived leukemia (cyclophilin b), melanoma (p15 protein, gp75, oncofetal antigen, GM2 and GD2 gangliosides), myeloma (MUC family; p21ras), non-small cell lung carcinoma (HER2/neu; c-erbB-2), nasopharyngeal cancer (Imp-1; EBNA-1), ovarian cancer (MUC family; HER2/neu; c-erbB-2), prostate cancer (Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3; PSMA; HER2/neu; c-erbB-2), pancreatic cancer (p21ras; MUC family; HER2/neu; c-erbB-2; ga733 glycoprotein), renal cancer (HER2/neu; c-erbB-2), squamous cell cancers of cervix and esophagus (viral products such as human papilloma virus proteins), testicular cancer (NY-ESO-1), T-cell leukemia (HTLV-1 epitopes), and melanoma (Melan-A/MART-1; cdc27; MAGE-3; p21ras; gp100<sup>Pmel117</sup>).

For examples of tumor antigens which bind to either or both MHC class I and MHC class II molecules, see the following references: Coulie, *Stem Cells* 13:393-403, 1995; Traversari et al. *J Exp Med* 176:1453-1457, 1992; Chaux et al. *J Immunol* 163:2928-2936, 1999; Fujie et al. *Int J Cancer* 80:169-172, 1999; Tanzarella et al. *Cancer Res* 59:2668-2674, 1999; van der Bruggen et al. *Eur J Immunol* 24:2134-2140, 1994; Chaux et al. *J Exp Med* 189:767-778, 1999; Kawashima et al. *Hum Immunol* 59:1-14, 1998; Tahara et al. *Clin Cancer Res* 5:2236-2241, 1999; Gaugler et al. *J Exp Med* 179:921-930, 1994; van der Bruggen et al. *Eur J Immunol* 24:3038-3043, 1994; Tanaka et al. *Cancer Res* 57:4465-4468, 1997; Oiso et al. *Int J Cancer* 81:387-394, 1999; Herman et al. *Immunogenetics* 43:377-383, 1996; Manici et al. *J Exp Med* 189:871-876, 1999; Duffour et al. *Eur J Immunol* 29:3329-3337, 1999; Zorn et al. *Eur J Immunol* 29:602-607, 1999; Huang et al. *J Immunol* 162:6849-6854, 1999; Boël et

- 64 -

al. *Immunity* 2:167-175, 1995; Van den Eynde et al. *J Exp Med* 182:689-698, 1995; De Backer et al. *Cancer Res* 59:3157-3165, 1999; Jäger et al. *J Exp Med* 187:265-270, 1998; Wang et al. *J Immunol* 161:3596-3606, 1998; Aarnoudse et al. *Int J Cancer* 82:442-448, 1999; Guilloux et al. *J Exp Med* 183:1173-1183, 1996; Lupetti et al. *J Exp Med* 188:1005-1016, 1998; Wölfel et al. *Eur J Immunol* 24:759-764, 1994; Skipper et al. *J Exp Med* 183:527-534, 1996; Kang et al. *J Immunol* 155:1343-1348, 1995; Morel et al. *Int J Cancer* 83:755-759, 1999; Brichard et al. *Eur J Immunol* 26:224-230, 1996; Kittlesen et al. *J Immunol* 160:2099-2106, 1998; Kawakami et al. *J Immunol* 161:6985-6992, 1998; Topalian et al. *J Exp Med* 183:1965-1971, 1996; Kobayashi et al. *Cancer Research* 58:296-301, 1998; Kawakami et al. *J Immunol* 154:3961-3968, 1995; Tsai et al. *J Immunol* 158:1796-1802, 1997; Cox et al. *Science* 264:716-719, 1994; Kawakami et al. *Proc Natl Acad Sci USA* 91:6458-6462, 1994; Skipper et al. *J Immunol* 157:5027-5033, 1996; Robbins et al. *J Immunol* 159:303-308, 1997; Castelli et al. *J Immunol* 162:1739-1748, 1999; Kawakami et al. *J Exp Med* 180:347-352, 1994; Castelli et al. *J Exp Med* 181:363-368, 1995; Schneider et al. *Int J Cancer* 75:451-458, 1998; Wang et al. *J Exp Med* 183:1131-1140, 1996; Wang et al. *J Exp Med* 184:2207-2216, 1996; Parkhurst et al. *Cancer Research* 58:4895-4901, 1998; Tsang et al. *J Natl Cancer Inst* 87:982-990, 1995; Correale et al. *J Natl Cancer Inst* 89:293-300, 1997; Coulie et al. *Proc Natl Acad Sci USA* 92:7976-7980, 1995; Wölfel et al. *Science* 269:1281-1284, 1995; Robbins et al. *J Exp Med* 183:1185-1192, 1996; Brändle et al. *J Exp Med* 183:2501-2508, 1996; ten Bosch et al. *Blood* 88:3522-3527, 1996; Mandruzzato et al. *J Exp Med* 186:785-793, 1997; Guéguen et al. *J Immunol* 160:6188-6194, 1998; Gjertsen et al. *Int J Cancer* 72:784-790, 1997; Gaudin et al. *J Immunol* 162:1730-1738, 1999; Chiari et al. *Cancer Res* 59:5785-5792, 1999; Hogan et al. *Cancer Res* 58:5144-5150, 1998; Pieper et al. *J Exp Med* 189:757-765, 1999; Wang et al. *Science* 284:1351-1354, 1999; Fisk et al. *J Exp Med* 181:2109-2117, 1995; Brossart et al. *Cancer Res* 58:732-736, 1998; Röpke et al. *Proc Natl Acad Sci USA* 93:14704-14707, 1996; Ikeda et al. *Immunity* 6:199-208, 1997; Ronsin et al. *J Immunol* 163:483-490, 1999; Vonderheide et al. *Immunity* 10:673-679, 1999. These antigens as well as others are disclosed in PCT Application PCT/US98/18601

The compositions and methods of the invention can be used alone or in conjunction with other agents and methods useful for the treatment of cancer. Cancer is currently treated using a variety of modalities including surgery, radiation therapy and chemotherapy. The choice of treatment modality will depend upon the type, location and dissemination of the

- 65 -

cancer. For example, surgery and radiation therapy may be more appropriate in the case of solid, well-defined tumor masses and less practical in the case of non-solid tumor cancers such as leukemia and lymphoma. One of the advantages of surgery and radiation therapy is the ability to control to some extent the impact of the therapy, and thus to limit the toxicity to normal tissues in the body. However, surgery and radiation therapy are often followed by chemotherapy to guard against any remaining or radio-resistant cancer cells. Chemotherapy is also the most appropriate treatment for disseminated cancers such as leukemia and lymphoma as well as metastases.

Chemotherapy refers to therapy using chemical and/or biological agents to attack cancer cells. Unlike localized surgery or radiation, chemotherapy is generally administered in a systemic fashion and thus toxicity to normal tissues is a major concern. Because many chemotherapy agents target cancer cells based on their proliferative profiles, tissues such as the gastrointestinal tract and the bone marrow which are normally proliferative are also susceptible to the effects of the chemotherapy. One of the major side effects of chemotherapy is myelosuppression (including anemia, neutropenia and thrombocytopenia) which results from the death of normal hemopoietic precursors.

Many chemotherapeutic agents have been developed for the treatment of cancer. Not all tumors, however, respond to chemotherapeutic agents and others although initially responsive to chemotherapeutic agents may develop resistance. As a result, the search for effective anti-cancer drugs has intensified in an effort to find even more effective agents with less non-specific toxicity.

Cancer medicaments function in a variety of ways. Some cancer medicaments work by targeting physiological mechanisms that are specific to tumor cells. Examples include the targeting of specific genes and their gene products (i.e., proteins primarily) which are mutated in cancers. Such genes include but are not limited to oncogenes (e.g., Ras, Her2, bcl-2), tumor suppressor genes (e.g., EGF, p53, Rb), and cell cycle targets (e.g., CDK4, p21, telomerase). Cancer medicaments can alternately target signal transduction pathways and molecular mechanisms which are altered in cancer cells. Targeting of cancer cells via the epitopes expressed on their cell surface is accomplished through the use of monoclonal antibodies. This latter type of cancer medicament is generally referred to herein as immunotherapy.

- 66 -

Other cancer medicaments target cells other than cancer cells. For example, some medicaments prime the immune system to attack tumor cells (i.e., cancer vaccines). Still other medicaments, called angiogenesis inhibitors, function by attacking the blood supply of solid tumors. Since the most malignant cancers are able to metastasize (i.e., exit the primary tumor site and seed a another site, thereby forming a secondary tumor), medicaments that  
5 impede this metastasis are also useful in the treatment of cancer. Angiogenic mediators include basic FGF, VEGF, angiopoietins, angiostatin, endostatin, TNF- $\alpha$ , TNP-470, thrombospondin-1, platelet factor 4, CAI, and certain members of the integrin family of proteins. One category of this type of medicament is a metalloproteinase inhibitor, which  
10 inhibits the enzymes used by the cancer cells to exist the primary tumor site and extravasate into another tissue.

Some cancer cells are antigenic and thus can be targeted by the immune system. In one aspect, the combined administration of nucleic acid and cancer medicaments, particularly those which are classified as cancer immunotherapies, is useful for stimulating a specific  
15 immune response against a cancer antigen.

The theory of immune surveillance is that a prime function of the immune system is to detect and eliminate neoplastic cells before a tumor forms. A basic principle of this theory is that cancer cells are antigenically different from normal cells and thus elicit immune reactions that are similar to those that cause rejection of immunologically incompatible  
20 allografts. Studies have confirmed that tumor cells differ, either qualitatively or quantitatively, in their expression of antigens. For example, "tumor-specific antigens" are antigens that are specifically associated with tumor cells but not normal cells. Examples of tumor specific antigens are viral antigens in tumors induced by DNA or RNA viruses. "Tumor-associated" antigens are present in both tumor cells and normal cells but are present  
25 in a different quantity or a different form in tumor cells. Examples of such antigens are oncofetal antigens (e.g., carcinoembryonic antigen), differentiation antigens (e.g., T and Tn antigens), and oncogene products (e.g., HER/neu).

Different types of cells that can kill tumor targets *in vitro* and *in vivo* have been identified: natural killer (NK) cells, cytolytic T lymphocytes (CTLs), lymphokine-activated  
30 killer cells (LAKs), and activated macrophages. NK cells can kill tumor cells without having been previously sensitized to specific antigens, and the activity does not require the presence of class I antigens encoded by the major histocompatibility complex (MHC) on target cells.

- 67 -

NK cells are thought to participate in the control of nascent tumors and in the control of metastatic growth. In contrast to NK cells, CTLs can kill tumor cells only after they have been sensitized to tumor antigens and when the target antigen is expressed on the tumor cells that also express MHC class I. CTLs are thought to be effector cells in the rejection of  
5 transplanted tumors and of tumors caused by DNA viruses. LAK cells are a subset of null lymphocytes distinct from the NK and CTL populations. Activated macrophages can kill tumor cells in a manner that is neither antigen-dependent nor MHC-restricted once activated. Activated macrophages are thought to decrease the growth rate of the tumors they infiltrate. *In vitro* assays have identified other immune mechanisms such as antibody-dependent, cell-  
10 mediated cytotoxic reactions and lysis by antibody plus complement. However, these immune effector mechanisms are thought to be less important *in vivo* than the function of NK, CTLs, LAK, and macrophages *in vivo* (for review see Piessens WF et al. "Tumor Immunology", In: Scientific American Medicine, Vol. 2, Scientific American Books, N.Y., pp. 1-13, 1996).

15 The goal of immunotherapy is to augment a patient's immune response to an established tumor. One method of immunotherapy includes the use of adjuvants. Adjuvant substances derived from microorganisms, such as bacillus Calmette-Guérin, heighten the immune response and enhance resistance to tumors in animals.

Immunotherapeutic agents are medicaments which derive from antibodies or antibody  
20 fragments which specifically bind or recognize a cancer antigen. Antibody-based immunotherapies may function by binding to the cell surface of a cancer cell and thereby stimulate the endogenous immune system to attack the cancer cell. Another way in which antibody-based therapy functions is as a delivery system for the specific targeting of toxic substances to cancer cells. Antibodies are usually conjugated to toxins such as ricin (e.g.,  
25 from castor beans), calicheamicin and maytansinoids, to radioactive isotopes such as Iodine-131 and Yttrium-90, to chemotherapeutic agents (as described herein), or to biological response modifiers. In this way, the toxic substances can be concentrated in the region of the cancer and non-specific toxicity to normal cells can be minimized. In addition to the use of antibodies which are specific for cancer antigens, antibodies which bind to vasculature, such  
30 as those which bind to endothelial cells, are also useful in the invention. This is because solid tumors generally are dependent upon newly formed blood vessels to survive, and thus most tumors are capable of recruiting and stimulating the growth of new blood vessels. As a

- 68 -

result, one strategy of many cancer medicaments is to attack the blood vessels feeding a tumor and/or the connective tissues (or stroma) supporting such blood vessels.

Cancer vaccines are medicaments which are intended to stimulate an endogenous immune response against cancer cells. Currently produced vaccines predominantly activate the humoral immune system (i.e., the antibody-dependent immune response). Other vaccines currently in development are focused on activating the cell-mediated immune system including cytotoxic T lymphocytes which are capable of killing tumor cells. Cancer vaccines generally enhance the presentation of cancer antigens to both antigen presenting cells (e.g., macrophages and dendritic cells) and/or to other immune cells such as T cells, B cells, and NK cells.

Although cancer vaccines may take one of several forms, as discussed infra, their purpose is to deliver cancer antigens and/or cancer associated antigens to antigen presenting cells (APC) in order to facilitate the endogenous processing of such antigens by APC and the ultimate presentation of antigen presentation on the cell surface in the context of MHC class I molecules. One form of cancer vaccine is a whole cell vaccine which is a preparation of cancer cells which have been removed from a subject, treated ex vivo and then reintroduced as whole cells in the subject. Lysates of tumor cells can also be used as cancer vaccines to elicit an immune response. Another form cancer vaccine is a peptide vaccine which uses cancer-specific or cancer-associated small proteins to activate T cells. Cancer-associated proteins are proteins which are not exclusively expressed by cancer cells (i.e., other normal cells may still express these antigens). However, the expression of cancer-associated antigens is generally consistently upregulated with cancers of a particular type. Other cancer vaccines include ganglioside vaccines, heat-shock protein vaccines, viral and bacterial vaccines, and nucleic acid vaccines.

Yet another form of cancer vaccine is a dendritic cell vaccine which includes whole dendritic cells which have been exposed to a cancer antigen or a cancer-associated antigen in vitro. Lysates or membrane fractions of dendritic cells may also be used as cancer vaccines. Dendritic cell vaccines are able to activate APCs directly. A dendritic cell is a professional APC. Dendritic cells form the link between the innate and the acquired immune system by presenting antigens and through their expression of pattern recognition receptors which detect microbial molecules like LPS in their local environment. Dendritic cells efficiently internalize, process, and present soluble specific antigen to which it is exposed. The process

- 69 -

of internalizing and presenting antigen causes rapid upregulation of the expression of major histocompatibility complex (MHC) and costimulatory molecules, the production of cytokines, and migration toward lymphatic organs where they are believed to be involved in the activation of T cells.

5           As used herein, chemotherapeutic agents embrace all other forms of cancer medicaments which do not fall into the categories of immunotherapeutic agents or cancer vaccines. Chemotherapeutic agents as used herein encompass both chemical and biological agents. These agents function to inhibit a cellular activity which the cancer cell is dependent upon for continued survival. Categories of chemotherapeutic agents include  
10 alkylating/alkaloid agents, antimetabolites, hormones or hormone analogs, and miscellaneous antineoplastic drugs. Most if not all of these agents are directly toxic to cancer cells and do not require immune stimulation.

          An "infectious disease" or, equivalently, an "infection" as used herein, refers to a disorder arising from the invasion of a host, superficially, locally, or systemically, by an  
15 infectious organism. Infectious organisms include bacteria, viruses, fungi, and parasites. Accordingly, "infectious disease" includes bacterial infections, viral infections, fungal infections and parasitic infections.

          A subject having an infectious disease is a subject that has been exposed to an infectious organism and has acute or chronic detectable levels of the organism in the body.  
20 Exposure to the infectious organism generally occurs with the external surface of the subject, e.g., skin or mucosal membranes and/or refers to the penetration of the external surface of the subject by the infectious organism.

          A subject at risk of developing an infectious disease is a subject who has a higher than normal risk of exposure to an infection causing pathogen. For instance, a subject at risk may  
25 be a subject who is planning to travel to an area where a particular type of infectious agent is found or it may be a subject who through lifestyle or medical procedures is exposed to bodily fluids which may contain infectious organisms or directly to the organism or a subject living in an area where an infectious organism has been identified. Subjects at risk of developing an infectious disease also include general populations to which a medical agency recommends  
30 vaccination against a particular infectious organism.

          A subject at risk of developing an infectious disease includes those subjects that have a general risk of exposure to a microorganism, e.g., influenza, but that do not have the active

- 70 -

disease during the treatment of the invention, as well as subjects that are considered to be at specific risk of developing an infectious disease because of medical or environmental factors that expose the subject to a particular microorganism.

Bacteria are unicellular organisms which multiply asexually by binary fission. They are classified and named based on their morphology, staining reactions, nutrition and metabolic requirements, antigenic structure, chemical composition, and genetic homology. Bacteria can be classified into three groups based on their morphological forms, spherical (coccus), straight-rod (bacillus) and curved or spiral rod (vibrio, campylobacter, spirillum, and spirochaete). Bacteria are also more commonly characterized based on their staining reactions into two classes of organisms, gram-positive and gram-negative. Gram refers to the method of staining which is commonly performed in microbiology labs. Gram-positive organisms retain the stain following the staining procedure and appear a deep violet color. Gram-negative organisms do not retain the stain but take up the counter-stain and thus appear pink.

Infectious bacteria include, but are not limited to, gram negative and gram positive bacteria. Gram positive bacteria include, but are not limited to *Pasteurella* species, *Staphylococci* species, and *Streptococcus* species. Gram negative bacteria include, but are not limited to, *Escherichia coli*, *Pseudomonas* species, and *Salmonella* species. Specific examples of infectious bacteria include but are not limited to: *Helicobacter pyloris*, *Borrelia burgdorferi*, *Legionella pneumophila*, *Mycobacteria* sps (e.g., *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. gordonae*), *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Streptococcus pyogenes* (Group A *Streptococcus*), *Streptococcus agalactiae* (Group B *Streptococcus*), *Streptococcus* (viridans group), *Streptococcus faecalis*, *Streptococcus bovis*, *Streptococcus* (anaerobic species), *Streptococcus pneumoniae*, pathogenic *Campylobacter* sp., *Enterococcus* sp., *Haemophilus influenzae*, *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Corynebacterium* sp., *Erysipelothrix rhusiopathiae*, *Clostridium perfringens*, *Clostridium tetani*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pasteurella multocida*, *Bacteroides* sp., *Fusobacterium nucleatum*, *Streptobacillus moniliformis*, *Treponema pallidum*, *Treponema pertenue*, *Leptospira*, *Rickettsia*, and *Actinomyces israeli*.

Viruses are small infectious agents which generally contain a nucleic acid core and a protein coat, but are not independently living organisms. Viruses can also take the form of

- 71 -

infectious nucleic acids lacking a protein. A virus cannot survive in the absence of a living cell within which it can replicate. Viruses enter specific living cells either by endocytosis or direct injection of DNA (phage) and multiply, causing disease. The multiplied virus can then be released and infect additional cells. Some viruses are DNA-containing viruses and others  
 5 are RNA-containing viruses. In some aspects, the invention also intends to treat diseases in which prions are implicated in disease progression such as for example bovine spongiform encephalopathy (i.e., mad cow disease, BSE) or scrapie infection in animals, or Creutzfeldt-Jakob disease in humans.

Viruses include, but are not limited to, enteroviruses (including, but not limited to,  
 10 viruses that the family *picornaviridae*, such as polio virus, coxsackie virus, echo virus), rotaviruses, adenovirus, hepatitis virus. Specific examples of viruses that have been found in humans include but are not limited to: *Retroviridae* (e.g., human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; *Picornaviridae* (e.g., polio viruses, hepatitis A virus; enteroviruses,  
 15 human Coxsackie viruses, rhinoviruses, echoviruses); *Calciviridae* (e.g., strains that cause gastroenteritis); *Togaviridae* (e.g., equine encephalitis viruses, rubella viruses); *Flaviviridae* (e.g., dengue viruses, encephalitis viruses, yellow fever viruses); *Coronaviridae* (e.g., coronaviruses); *Rhabdoviridae* (e.g., vesicular stomatitis viruses, rabies viruses); *Filoviridae* (e.g., ebola viruses); *Paramyxoviridae* (e.g., parainfluenza viruses, mumps virus, measles  
 20 virus, respiratory syncytial virus); *Orthomyxoviridae* (e.g., influenza viruses); *Bunyaviridae* (e.g., Hantaan viruses, bunga viruses, phleboviruses and Nairo viruses); *Arenaviridae* (hemorrhagic fever viruses); *Reoviridae* (e.g., reoviruses, orbiviruses and rotaviruses); *Birnaviridae*; *Hepadnaviridae* (Hepatitis B virus); *Parvoviridae* (parvoviruses); *Papovaviridae* (papillomaviruses, polyoma viruses); *Adenoviridae* (most adenoviruses);  
 25 *Herpesviridae* (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV)); *Poxviridae* (variola viruses, vaccinia viruses, pox viruses); *Iridoviridae* (e.g., African swine fever virus); and unclassified viruses (e.g., the etiological agents of spongiform encephalopathies, the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1 = internally transmitted; class 2 =  
 30 parenterally transmitted (i.e., Hepatitis C); Norwalk and related viruses, and astroviruses).

Fungi are eukaryotic organisms, only a few of which cause infection in vertebrate mammals. Because fungi are eukaryotic organisms, they differ significantly from

- 72 -

prokaryotic bacteria in size, structural organization, life cycle and mechanism of multiplication. Fungi are classified generally based on morphological features, modes of reproduction and culture characteristics. Although fungi can cause different types of disease in subjects, such as respiratory allergies following inhalation of fungal antigens, fungal  
5 intoxication due to ingestion of toxic substances, such as *Amanita phalloides* toxin and phallotoxin produced by poisonous mushrooms and aflatoxins, produced by aspergillus species, not all fungi cause infectious disease.

Infectious fungi can cause systemic or superficial infections. Primary systemic infection can occur in normal healthy subjects, and opportunistic infections are most  
10 frequently found in immunocompromised subjects. The most common fungal agents causing primary systemic infection include *Blastomyces*, *Coccidioides*, and *Histoplasma*. Common fungi causing opportunistic infection in immunocompromised or immunosuppressed subjects include, but are not limited to, *Candida albicans*, *Cryptococcus neoformans*, and various *Aspergillus* species. Systemic fungal infections are invasive infections of the internal organs.  
15 The organism usually enters the body through the lungs, gastrointestinal tract, or intravenous catheters. These types of infections can be caused by primary pathogenic fungi or opportunistic fungi.

Superficial fungal infections involve growth of fungi on an external surface without invasion of internal tissues. Typical superficial fungal infections include cutaneous fungal  
20 infections involving skin, hair, or nails.

Diseases associated with fungal infection include aspergillosis, blastomycosis, candidiasis, chromoblastomycosis, coccidioidomycosis, cryptococcosis, fungal eye infections, fungal hair, nail, and skin infections, histoplasmosis, lobomycosis, mycetoma, otomycosis, paracoccidioidomycosis, disseminated *Penicillium marneffei*,  
25 phaeohyphomycosis, rhinosporidiosis, sporotrichosis, and zygomycosis.

Parasites are organisms which depend upon other organisms in order to survive and thus must enter, or infect, another organism to continue their life cycle. The infected organism, i.e., the host, provides both nutrition and habitat to the parasite. Although in its broadest sense the term parasite can include all infectious agents (i.e., bacteria, viruses, fungi,  
30 protozoa and helminths), generally speaking, the term is used to refer solely to protozoa, helminths, and ectoparasitic arthropods (e.g., ticks, mites, etc.). Protozoa are single-celled organisms which can replicate both intracellularly and extracellularly, particularly in the

- 73 -

blood, intestinal tract or the extracellular matrix of tissues. Helminths are multicellular organisms which almost always are extracellular (an exception being *Trichinella* spp.). Helminths normally require exit from a primary host and transmission into a secondary host in order to replicate. In contrast to these aforementioned classes, ectoparasitic arthropods  
5 form a parasitic relationship with the external surface of the host body.

Parasites include intracellular parasites and obligate intracellular parasites. Examples of parasites include but are not limited to *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium knowlesi*, *Babesia microti*, *Babesia divergens*, *Trypanosoma cruzi*, *Toxoplasma gondii*, *Trichinella spiralis*, *Leishmania major*,  
10 *Leishmania donovani*, *Leishmania braziliensis*, *Leishmania tropica*, *Trypanosoma gambiense*, *Trypanosoma rhodesiense* and *Schistosoma mansoni*.

Other medically relevant microorganisms have been described extensively in the literature, e.g., see C.G.A Thomas, *Medical Microbiology*, Bailliere Tindall, Great Britain 1983, the entire contents of which is hereby incorporated by reference. Each of the foregoing  
15 lists is illustrative and is not intended to be limiting.

The compositions and methods of the invention can be used alone or in conjunction with other agents and methods useful for the treatment of infection. Infection medicaments include but are not limited to anti-bacterial agents, anti-viral agents, anti-fungal agents and anti-parasitic agents. Phrases such as "anti-infective agent", "antibiotic", "anti-bacterial  
20 agent", "anti-viral agent", "anti-fungal agent", "anti-parasitic agent" and "parasiticide" have well-established meanings to those of ordinary skill in the art and are defined in standard medical texts. Briefly, anti-bacterial agents kill or inhibit bacteria, and include antibiotics as well as other synthetic or natural compounds having similar functions. Anti-viral agents can be isolated from natural sources or synthesized and are useful for killing or inhibiting viruses.  
25 Anti-fungal agents are used to treat superficial fungal infections as well as opportunistic and primary systemic fungal infections. Anti-parasite agents kill or inhibit parasites. Many antibiotics are low molecular weight molecules which are produced as secondary metabolites by cells, such as microorganisms. In general, antibiotics interfere with one or more functions or structures which are specific for the microorganism and which are not present in host cells.

30 One of the problems with anti-infective therapies is the side effects occurring in the host that is treated with the anti-infective agent. For instance, many anti-infectious agents can kill or inhibit a broad spectrum of microorganisms and are not specific for a particular

- 74 -

type of species. Treatment with these types of anti-infectious agents results in the killing of the normal microbial flora living in the host, as well as the infectious microorganism. The loss of the microbial flora can lead to disease complications and predispose the host to infection by other pathogens, since the microbial flora compete with and function as barriers to infectious pathogens. Other side effects may arise as a result of specific or non-specific effects of these chemical entities on non-microbial cells or tissues of the host.

Another problem with widespread use of anti-infectants is the development of antibiotic-resistant strains of microorganisms. Already, vancomycin-resistant *enterococci*, penicillin-resistant *pneumococci*, multi-resistant *S. aureus*, and multi-resistant *tuberculosis* strains have developed and are becoming major clinical problems. Widespread use of anti-infectants will likely produce many antibiotic-resistant strains of bacteria. As a result, new anti-infective strategies will be required to combat these microorganisms.

Antibacterial antibiotics which are effective for killing or inhibiting a wide range of bacteria are referred to as broad-spectrum antibiotics. Other types of antibacterial antibiotics are predominantly effective against the bacteria of the class gram-positive or gram-negative. These types of antibiotics are referred to as narrow-spectrum antibiotics. Other antibiotics which are effective against a single organism or disease and not against other types of bacteria, are referred to as limited-spectrum antibiotics.

Anti-bacterial agents are sometimes classified based on their primary mode of action. In general, anti-bacterial agents are cell wall synthesis inhibitors, cell membrane inhibitors, protein synthesis inhibitors, nucleic acid synthesis or functional inhibitors, and competitive inhibitors. Cell wall synthesis inhibitors inhibit a step in the process of cell wall synthesis, and in general in the synthesis of bacterial peptidoglycan. Cell wall synthesis inhibitors include  $\beta$ -lactam antibiotics, natural penicillins, semi-synthetic penicillins, ampicillin, clavulanic acid, cephalosporins, and bacitracin.

The  $\beta$ -lactams are antibiotics containing a four-membered  $\beta$ -lactam ring which inhibits the last step of peptidoglycan synthesis.  $\beta$ -lactam antibiotics can be synthesized or natural. The  $\beta$ -lactam antibiotics produced by *penicillium* are the natural penicillins, such as penicillin G or penicillin V. These are produced by fermentation of *Penicillium chrysogenum*. The natural penicillins have a narrow spectrum of activity and are generally effective against *Streptococcus*, *Gonococcus*, and *Staphylococcus*. Other types of natural

- 75 -

penicillins, which are also effective against gram-positive bacteria, include penicillins F, X, K, and O.

Semi-synthetic penicillins are generally modifications of the molecule 6-aminopenicillanic acid produced by a mold. The 6-aminopenicillanic acid can be modified  
5 by addition of side chains which produce penicillins having broader spectrums of activity than natural penicillins or various other advantageous properties. Some types of semi-synthetic penicillins have broad spectrums against gram-positive and gram-negative bacteria, but are inactivated by penicillinase. These semi-synthetic penicillins include ampicillin, carbenicillin, oxacillin, azlocillin, mezlocillin, and piperacillin. Other types of semi-synthetic  
10 penicillins have narrower activities against gram-positive bacteria, but have developed properties such that they are not inactivated by penicillinase. These include, for instance, methicillin, dicloxacillin, and nafcillin. Some of the broad spectrum semi-synthetic penicillins can be used in combination with  $\beta$ -lactamase inhibitors, such as clavulanic acids and sulbactam. The  $\beta$ -lactamase inhibitors do not have anti-microbial action but they  
15 function to inhibit penicillinase, thus protecting the semi-synthetic penicillin from degradation.

One of the serious side effects associated with penicillins, both natural and semi-synthetic, is penicillin allergy. Penicillin allergies are very serious and can cause death rapidly. In a subject that is allergic to penicillin, the  $\beta$ -lactam molecule will attach to a serum  
20 protein which initiates an IgE-mediated inflammatory response. The inflammatory response leads to anaphylaxis and possibly death.

Another type of  $\beta$ -lactam antibiotic is the cephalosporins. They are sensitive to degradation by bacterial  $\beta$ -lactamases, and thus, are not always effective alone. Cephalosporins, however, are resistant to penicillinase. They are effective against a variety  
25 of gram-positive and gram-negative bacteria. Cephalosporins include, but are not limited to, cephalothin, cephapirin, cephalixin, cefamandole, cefaclor, cefazolin, cefuroxime, cefoxitin, cefotaxime, cefsulodin, cefetamet, cefixime, ceftriaxone, cefoperazone, ceftazidime, and moxalactam.

Bacitracin is another class of antibiotics which inhibit cell wall synthesis, by  
30 inhibiting the release of muropeptide subunits or peptidoglycan from the molecule that delivers the subunit to the outside of the membrane. Although bacitracin is effective against

- 76 -

gram-positive bacteria, its use is limited in general to topical administration because of its high toxicity.

Carbapenems are another broad-spectrum  $\beta$ -lactam antibiotic, which is capable of inhibiting cell wall synthesis. Examples of carbapenems include, but are not limited to, imipenems. Monobactams are also broad-spectrum  $\beta$ -lactam antibiotics, and include, euztreonam. An antibiotic produced by *Streptomyces*, vancomycin, is also effective against gram-positive bacteria by inhibiting cell membrane synthesis.

Another class of anti-bacterial agents is the anti-bacterial agents that are cell membrane inhibitors. These compounds disorganize the structure or inhibit the function of bacterial membranes. One problem with anti-bacterial agents that are cell membrane inhibitors is that they can produce effects in eukaryotic cells as well as bacteria because of the similarities in phospholipids in bacterial and eukaryotic membranes. Thus these compounds are rarely specific enough to permit these compounds to be used systemically and prevent the use of high doses for local administration.

One clinically useful cell membrane inhibitor is Polymyxin. Polymyxins interfere with membrane function by binding to membrane phospholipids. Polymyxin is effective mainly against Gram-negative bacteria and is generally used in severe *Pseudomonas* infections or *Pseudomonas* infections that are resistant to less toxic antibiotics. The severe side effects associated with systemic administration of this compound include damage to the kidney and other organs.

Other cell membrane inhibitors include Amphotericin B and Nystatin which are anti-fungal agents used predominantly in the treatment of systemic fungal infections and *Candida* yeast infections. Imidazoles are another class of antibiotic that is a cell membrane inhibitor. Imidazoles are used as anti-bacterial agents as well as anti-fungal agents, e.g., used for treatment of yeast infections, dermatophytic infections, and systemic fungal infections. Imidazoles include but are not limited to clotrimazole, miconazole, ketoconazole, itraconazole, and fluconazole.

Many anti-bacterial agents are protein synthesis inhibitors. These compounds prevent bacteria from synthesizing structural proteins and enzymes and thus cause inhibition of bacterial cell growth or function or cell death. In general these compounds interfere with the processes of transcription or translation. Anti-bacterial agents that block transcription include but are not limited to Rifampins and Ethambutol. Rifampins, which inhibit the enzyme RNA

- 77 -

polymerase, have a broad spectrum activity and are effective against gram-positive and gram-negative bacteria as well as *Mycobacterium tuberculosis*. Ethambutol is effective against *Mycobacterium tuberculosis*.

Anti-bacterial agents which block translation interfere with bacterial ribosomes to prevent mRNA from being translated into proteins. In general this class of compounds includes but is not limited to tetracyclines, chloramphenicol, the macrolides (e.g., erythromycin) and the aminoglycosides (e.g., streptomycin).

The aminoglycosides are a class of antibiotics which are produced by the bacterium *Streptomyces*, such as, for instance streptomycin, kanamycin, tobramycin, amikacin, and gentamicin. Aminoglycosides have been used against a wide variety of bacterial infections caused by Gram-positive and Gram-negative bacteria. Streptomycin has been used extensively as a primary drug in the treatment of *tuberculosis*. Gentamicin is used against many strains of Gram-positive and Gram-negative bacteria, including *Pseudomonas* infections, especially in combination with Tobramycin. Kanamycin is used against many Gram-positive bacteria, including penicillin-resistant *Staphylococci*. One side effect of aminoglycosides that has limited their use clinically is that at dosages which are essential for efficacy, prolonged use has been shown to impair kidney function and cause damage to the auditory nerves leading to deafness.

Another type of translation inhibitor anti-bacterial agent is the tetracyclines. The tetracyclines are a class of antibiotics that are broad-spectrum and are effective against a variety of gram-positive and gram-negative bacteria. Examples of tetracyclines include tetracycline, minocycline, doxycycline, and chlortetracycline. They are important for the treatment of many types of bacteria but are particularly important in the treatment of Lyme disease. As a result of their low toxicity and minimal direct side effects, the tetracyclines have been overused and misused by the medical community, leading to problems. For instance, their overuse has led to widespread development of resistance.

Anti-bacterial agents such as the macrolides bind reversibly to the 50 S ribosomal subunit and inhibit elongation of the protein by peptidyl transferase or prevent the release of uncharged tRNA from the bacterial ribosome or both. These compounds include erythromycin, roxithromycin, clarithromycin, oleandomycin, and azithromycin. Erythromycin is active against most Gram-positive bacteria, *Neisseria*, *Legionella* and *Haemophilus*, but not against the *Enterobacteriaceae*. Lincomycin and clindamycin, which

- 78 -

block peptide bond formation during protein synthesis, are used against gram-positive bacteria.

Another type of translation inhibitor is chloramphenicol. Chloramphenicol binds the 70 S ribosome inhibiting the bacterial enzyme peptidyl transferase thereby preventing the growth of the polypeptide chain during protein synthesis. One serious side effect associated with chloramphenicol is aplastic anemia. Aplastic anemia develops at doses of chloramphenicol which are effective for treating bacteria in a small proportion (1/50,000) of patients. Chloramphenicol which was once a highly prescribed antibiotic is now seldom used as a result of the deaths from anemia. Because of its effectiveness it is still used in life-threatening situations (e.g., typhoid fever).

Some anti-bacterial agents disrupt nucleic acid synthesis or function, e.g., bind to DNA or RNA so that their messages cannot be read. These include but are not limited to quinolones and co-trimoxazole, both synthetic chemicals and rifamycins, a natural or semi-synthetic chemical. The quinolones block bacterial DNA replication by inhibiting the DNA gyrase, the enzyme needed by bacteria to produce their circular DNA. They are broad spectrum and examples include norfloxacin, ciprofloxacin, enoxacin, nalidixic acid and temafloxacin. Nalidixic acid is a bactericidal agent that binds to the DNA gyrase enzyme (topoisomerase) which is essential for DNA replication and allows supercoils to be relaxed and reformed, inhibiting DNA gyrase activity. The main use of nalidixic acid is in treatment of lower urinary tract infections (UTI) because it is effective against several types of Gram-negative bacteria such as *E. coli*, *Enterobacter aerogenes*, *K. pneumoniae* and *Proteus* species which are common causes of UTI. Co-trimoxazole is a combination of sulfamethoxazole and trimethoprim, which blocks the bacterial synthesis of folic acid needed to make DNA nucleotides. Rifampicin is a derivative of rifamycin that is active against Gram-positive bacteria (including *Mycobacterium tuberculosis* and meningitis caused by *Neisseria meningitidis*) and some Gram-negative bacteria. Rifampicin binds to the beta subunit of the polymerase and blocks the addition of the first nucleotide which is necessary to activate the polymerase, thereby blocking mRNA synthesis.

Another class of anti-bacterial agents is compounds that function as competitive inhibitors of bacterial enzymes. The competitive inhibitors are mostly all structurally similar to a bacterial growth factor and compete for binding but do not perform the metabolic function in the cell. These compounds include sulfonamides and chemically modified forms

of sulfanilamide which have even higher and broader antibacterial activity. The sulfonamides (e.g., gantrisin and trimethoprim) are useful for the treatment of *Streptococcus pneumoniae*, beta-hemolytic *streptococci* and *E. coli*, and have been used in the treatment of uncomplicated UTI caused by *E. coli*, and in the treatment of meningococcal meningitis.

5       Anti-viral agents are compounds which prevent infection of cells by viruses or replication of the virus within the cell. There are many fewer antiviral drugs than antibacterial drugs because the process of viral replication is so closely related to DNA replication within the host cell, that non-specific antiviral agents would often be toxic to the host. There are several stages within the process of viral infection which can be blocked or  
10       inhibited by antiviral agents. These stages include, attachment of the virus to the host cell (immunoglobulin or binding peptides), uncoating of the virus (e.g. amantadine), synthesis or translation of viral mRNA (e.g. interferon), replication of viral RNA or DNA (e.g. nucleoside analogues), maturation of new virus proteins (e.g. protease inhibitors), and budding and release of the virus.

15       Another category of anti-viral agents are nucleoside analogues. Nucleoside analogues are synthetic compounds which are similar to nucleosides, but which have an incomplete or abnormal deoxyribose or ribose group. Once the nucleoside analogues are in the cell, they are phosphorylated, producing the triphosphate form which competes with normal nucleotides for incorporation into the viral DNA or RNA. Once the triphosphate form of the  
20       nucleoside analogue is incorporated into the growing nucleic acid chain, it causes irreversible association with the viral polymerase and thus chain termination. Nucleoside analogues include, but are not limited to, acyclovir (used for the treatment of herpes simplex virus and varicella-zoster virus), gancyclovir (useful for the treatment of cytomegalovirus), idoxuridine, ribavirin (useful for the treatment of respiratory syncytial virus), dideoxyinosine,  
25       dideoxycytidine, and zidovudine (azidothymidine).

Another class of anti-viral agents includes cytokines such as interferons. The interferons are cytokines which are secreted by virus-infected cells as well as immune cells. The interferons function by binding to specific receptors on cells adjacent to the infected cells, causing the change in the cell which protects it from infection by the virus.  $\alpha$  and  $\beta$ -  
30       interferon also induce the expression of Class I and Class II MHC molecules on the surface of infected cells, resulting in increased antigen presentation for host immune cell recognition.  $\alpha$  and  $\beta$ -interferons are available as recombinant forms and have been used for the treatment of

- 80 -

chronic hepatitis B and C infection. At the dosages which are effective for anti-viral therapy, interferons have severe side effects such as fever, malaise and weight loss.

Immunoglobulin therapy is used for the prevention of viral infection.

Immunoglobulin therapy for viral infections is different from bacterial infections, because  
5 rather than being antigen-specific, the immunoglobulin therapy functions by binding to  
extracellular virions and preventing them from attaching to and entering cells which are  
susceptible to the viral infection. The therapy is useful for the prevention of viral infection  
for the period of time that the antibodies are present in the host. In general there are two  
types of immunoglobulin therapies, normal immune globulin therapy and hyper-immune  
10 globulin therapy. Normal immune globulin therapy utilizes a antibody product which is  
prepared from the serum of normal blood donors and pooled. This pooled product contains  
low titers of antibody to a wide range of human viruses, such as hepatitis A, parvovirus,  
enterovirus (especially in neonates). Hyper-immune globulin therapy utilizes antibodies  
which are prepared from the serum of individuals who have high titers of an antibody to a  
15 particular virus. Those antibodies are then used against a specific virus. Examples of hyper-  
immune globulins include zoster immune globulin (useful for the prevention of varicella in  
immunocompromised children and neonates), human rabies immune globulin (useful in the  
post-exposure prophylaxis of a subject bitten by a rabid animal), hepatitis B immune globulin  
(useful in the prevention of hepatitis B virus, especially in a subject exposed to the virus), and  
20 RSV immune globulin (useful in the treatment of respiratory syncytial virus infections).

Anti-fungal agents are useful for the treatment and prevention of infective fungi.

Anti-fungal agents are sometimes classified by their mechanism of action. Some anti-fungal  
agents function as cell wall inhibitors by inhibiting glucose synthase. These include, but are  
not limited to, basiungin/ECB. Other anti-fungal agents function by destabilizing membrane  
25 integrity. These include, but are not limited to, imidazoles, such as clotrimazole,  
sertaconazole, fluconazole, itraconazole, ketoconazole, miconazole, and voriconazole, as well  
as FK 463, amphotericin B, BAY 38-9502, MK 991, pradimicin, UK 292, butenafine, and  
terbinafine. Other anti-fungal agents function by breaking down chitin (e.g., chitinase) or  
immunosuppression (501 cream).

30 Parasitocides are agents that kill parasites directly. Such compounds are known in the  
art and are generally commercially available. Examples of parasitocides useful for human  
administration include but are not limited to albendazole, amphotericin B, benznidazole,

- 81 -

bithionol, chloroquine HCl, chloroquine phosphate, clindamycin, dehydroemetine, diethylcarbamazine, diloxanide furoate, eflornithine, furazolidone, glucocorticoids, halofantrine, iodoquinol, ivermectin, mebendazole, mefloquine, meglumine antimoniate, melarsoprol, metrifonate, metronidazole, niclosamide, nifurtimox, oxamniquine, paromomycin, pentamidine isethionate, piperazine, praziquantel, primaquine phosphate, 5 proguanil, pyrantel pamoate, pyrimethamine-sulfonamides, pyrimethamine-sulfadoxine, quinacrine HCl, quinine sulfate, quinidine gluconate, spiramycin, stibogluconate sodium (sodium antimony gluconate), suramin, tetracycline, doxycycline, thiabendazole, tinidazole, trimethoprim-sulfamethoxazole, and tryparsamide.

10       The compositions and methods of the invention may also find use in the treatment of allergy and asthma.

      An "allergy" refers to acquired hypersensitivity to a substance (allergen). Allergic conditions include but are not limited to eczema, allergic rhinitis or coryza, hay fever, allergic conjunctivitis, bronchial asthma, urticaria (hives) and food allergies, other atopic conditions 15 including atopic dermatitis; anaphylaxis; drug allergy; and angioedema. Allergic diseases include but are not limited to rhinitis (hay fever), asthma, urticaria, and atopic dermatitis.

      Allergy is a disease associated with the production of antibodies from a particular class of immunoglobulin, IgE, against allergens. The development of an IgE-mediated response to common aeroallergens is also a factor which indicates predisposition towards the 20 development of asthma. If an allergen encounters a specific IgE which is bound to an IgE Fc receptor (FcεR) on the surface of a basophil (circulating in the blood) or mast cell (dispersed throughout solid tissue), the cell becomes activated, resulting in the production and release of mediators such as histamine, serotonin, and lipid mediators.

      A subject having an allergy is a subject that is currently experiencing or has 25 previously experienced an allergic reaction in response to an allergen.

      A subject at risk of developing an allergy or asthma is a subject that has been identified as having an allergy or asthma in the past but who is not currently experiencing the active disease, as well as a subject that is considered to be at risk of developing asthma or allergy because of genetic or environmental factors. A subject at risk of developing allergy 30 or asthma can also include a subject who has any risk of exposure to an allergen or a risk of developing asthma, i.e., someone who has suffered from an asthmatic attack previously or has a predisposition to asthmatic attacks. For instance, a subject at risk may be a subject who

- 82 -

is planning to travel to an area where a particular type of allergen or asthmatic initiator is found or it may even be any subject living in an area where an allergen has been identified. If the subject develops allergic responses to a particular antigen and the subject may be exposed to the antigen, i.e., during pollen season, then that subject is at risk of exposure to the antigen.

5       The generic name for molecules that cause an allergic reaction is allergen. An "allergen" as used herein is a molecule capable of provoking an immune response characterized by production of IgE. An allergen is a substance that can induce an allergic or asthmatic response in a susceptible subject. Thus, in the context of this invention, the term allergen means a specific type of antigen which can trigger an allergic response which is  
10       mediated by IgE antibody. The method and preparations of this invention extend to a broad class of such allergens and fragments of allergens or haptens acting as allergens. The list of allergens is enormous and can include pollens, insect venoms, animal dander, dust, fungal spores, and drugs (e.g., penicillin).

      There are numerous species of allergens. The allergic reaction occurs when tissue-  
15       sensitizing immunoglobulin of the IgE type reacts with foreign allergen. The IgE antibody is bound to mast cells and/or basophils, and these specialized cells release chemical mediators (vasoactive amines) of the allergic reaction when stimulated to do so by allergens bridging the ends of the antibody molecule. Histamine, platelet activating factor, arachidonic acid metabolites, and serotonin are among the best known mediators of allergic reactions in man.  
20       Histamine and the other vasoactive amines are normally stored in mast cells and basophil leukocytes. The mast cells are dispersed throughout animal tissue and the basophils circulate within the vascular system. These cells manufacture and store histamine within the cell unless the specialized sequence of events involving IgE binding occurs to trigger its release.

      The symptoms of the allergic reaction vary, depending on the location within the body  
25       where the IgE reacts with the antigen. If the reaction occurs along the respiratory epithelium, the symptoms are sneezing, coughing and asthmatic reactions. If the interaction occurs in the digestive tract, as in the case of food allergies, abdominal pain and diarrhea are common. Systemic reactions, for example following a bee sting, can be severe and often life-threatening.

30       Delayed-type hypersensitivity, also known as type IV allergy reaction, is an allergic reaction characterized by a delay period of at least 12 hours from invasion of the antigen into the allergic subject until appearance of the inflammatory or immune reaction. The T

- 83 -

lymphocytes (sensitized T lymphocytes) of individuals in an allergic condition react with the antigen, triggering the T lymphocytes to release lymphokines (macrophage migration inhibitory factor (MIF), macrophage activating factor (MAF), mitogenic factor (MF), skin-reactive factor (SRF), chemotactic factor, neovascularization-accelerating factor, etc.), which  
5 function as inflammation mediators, and the biological activity of these lymphokines, together with the direct and indirect effects of locally appearing lymphocytes and other inflammatory immune cells, give rise to the type IV allergy reaction. Delayed allergy reactions include tuberculin type reaction, homograft rejection reaction, cell-dependent type protective reaction, contact dermatitis hypersensitivity reaction, and the like, which are  
10 known to be most strongly suppressed by steroidal agents. Consequently, steroidal agents are effective against diseases which are caused by delayed allergy reactions. Long-term use of steroidal agents at concentrations currently being used can, however, lead to the serious side-effect known as steroid dependence. The methods of the invention solve some of these problems, by providing for lower and fewer doses to be administered.

15 Immediate hypersensitivity (or anaphylactic response) is a form of allergic reaction which develops very quickly, i.e., within seconds or minutes of exposure of the patient to the causative allergen, and it is mediated by IgE antibodies made by B lymphocytes. In nonallergic patients, there is no IgE antibody of clinical relevance; but, in a person suffering with allergic diseases, IgE antibody mediates immediate hypersensitivity by sensitizing mast  
20 cells which are abundant in the skin, lymphoid organs, in the membranes of the eye, nose and mouth, and in the respiratory tract and intestines.

Mast cells have surface receptors for IgE, and the IgE antibodies in allergy-suffering patients become bound to them. As discussed briefly above, when the bound IgE is subsequently contacted by the appropriate allergen, the mast cell is caused to degranulate and  
25 to release various substances called bioactive mediators, such as histamine, into the surrounding tissue. It is the biologic activity of these substances which is responsible for the clinical symptoms typical of immediate hypersensitivity; namely, contraction of smooth muscle in the airways or the intestine, the dilation of small blood vessels and the increase in their permeability to water and plasma proteins, the secretion of thick sticky mucus, and in  
30 the skin, redness, swelling and the stimulation of nerve endings that results in itching or pain.

"Asthma" as used herein refers to a disorder of the respiratory system characterized by inflammation, narrowing of the airways, and increased reactivity of the airways to inhaled

- 84 -

agents. Asthma is frequently, although not exclusively, associated with an atopic or allergic condition. Symptoms of asthma include recurrent episodes of wheezing, breathlessness, and chest tightness, and coughing, resulting from airflow obstruction. Airway inflammation associated with asthma can be detected through observation of a number of physiological changes, such as, denudation of airway epithelium, collagen deposition beneath basement membrane, edema, mast cell activation, inflammatory cell infiltration, including neutrophils, inosineophils, and lymphocytes. As a result of the airway inflammation, asthma patients often experience airway hyper-responsiveness, airflow limitation, respiratory symptoms, and disease chronicity. Airflow limitations include acute bronchoconstriction, airway edema, mucous plug formation, and airway remodeling, features which often lead to bronchial obstruction. In some cases of asthma, sub-basement membrane fibrosis may occur, leading to persistent abnormalities in lung function.

Research over the past several years has revealed that asthma likely results from complex interactions among inflammatory cells, mediators, and other cells and tissues resident in the airway. Mast cells, inosineophils, epithelial cells, macrophage, and activated T-cells all play an important role in the inflammatory process associated with asthma. Djukanovic R et al. (1990) *Am Rev Respir Dis* 142:434-457. It is believed that these cells can influence airway function through secretion of preformed and newly synthesized mediators which can act directly or indirectly on the local tissue. It has also been recognized that subpopulations of T-lymphocytes (Th2) play an important role in regulating allergic inflammation in the airway by releasing selective cytokines and establishing disease chronicity. Robinson DS et al. (1992) *N Engl J Med* 326:298-304.

Asthma is a complex disorder which arises at different stages in development and can be classified based on the degree of symptoms as acute, subacute or chronic. An acute inflammatory response is associated with an early recruitment of cells into the airway. The subacute inflammatory response involves the recruitment of cells as well as the activation of resident cells causing a more persistent pattern of inflammation. Chronic inflammatory response is characterized by a persistent level of cell damage and an ongoing repair process, which may result in permanent abnormalities in the airway.

A "subject having asthma" is a subject that has a disorder of the respiratory system characterized by inflammation, narrowing of the airways and increased reactivity of the airways to inhaled agents. Asthma is frequently, although not exclusively, associated with

- 85 -

atopic or allergic symptoms. An "initiator" as used herein refers to a composition or environmental condition which triggers asthma. Initiators include, but are not limited to, allergens, cold temperatures, exercise, viral infections, SO<sub>2</sub>.

The compositions and methods of the invention can be used alone or in conjunction  
5 with other agents and methods useful in the treatment of asthma. An "asthma/allergy  
medicament" as used herein is a composition of matter which reduces the symptoms of,  
prevents the development of, or inhibits an asthmatic or allergic reaction. Various types of  
medicaments for the treatment of asthma and allergy are described in the Guidelines For The  
Diagnosis and Management of Asthma, Expert Panel Report 2, NIH Publication No. 97/4051,  
10 July 19, 1997, the entire contents of which are incorporated herein by reference. The  
summary of the medicaments as described in the NIH publication is presented below. In  
most embodiments the asthma/allergy medicament is useful to some degree for treating both  
asthma and allergy.

Medications for the treatment of asthma are generally separated into two categories,  
15 quick-relief medications and long-term control medications. Asthma patients take the long-  
term control medications on a daily basis to achieve and maintain control of persistent  
asthma. Long-term control medications include anti-inflammatory agents such as  
corticosteroids, chromolyn sodium and nedocromil; long-acting bronchodilators, such as  
long-acting  $\beta_2$ -agonists and methylxanthines; and leukotriene modifiers. The quick-relief  
20 medications include short-acting  $\beta_2$  agonists, anti-cholinergics, and systemic corticosteroids.  
There are many side effects associated with each of these drugs and none of the drugs alone  
or in combination is capable of preventing or completely treating asthma.

Asthma medicaments include, but are not limited, PDE-4 inhibitors,  
bronchodilator/beta-2 agonists, K<sup>+</sup> channel openers, VLA-4 antagonists, neurokin  
25 antagonists, thromboxane A<sub>2</sub> (TXA<sub>2</sub>) synthesis inhibitors, xanthines, arachidonic acid  
antagonists, 5 lipoxygenase inhibitors, TXA<sub>2</sub> receptor antagonists, TXA<sub>2</sub> antagonists,  
inhibitor of 5-lipoxygenase activation proteins, and protease inhibitors.

Bronchodilator/ $\beta_2$  agonists are a class of compounds which cause bronchodilation or  
smooth muscle relaxation. Bronchodilator/ $\beta_2$  agonists include, but are not limited to,  
30 salmeterol, salbutamol, albuterol, terbutaline, D2522/formoterol, fenoterol, bitolterol,  
pirbuerol methylxanthines and orciprenaline. Long-acting  $\beta_2$  agonists and bronchodilators  
are compounds which are used for long-term prevention of symptoms in addition to the anti-

- 86 -

inflammatory therapies. Long-acting  $\beta_2$  agonists include, but are not limited to, salmeterol and albuterol. These compounds are usually used in combination with corticosteroids and generally are not used without any inflammatory therapy. They have been associated with side effects such as tachycardia, skeletal muscle tremor, hypokalemia, and prolongation of QTc interval in overdose.

Methylxanthines, including for instance theophylline, have been used for long-term control and prevention of symptoms. These compounds cause bronchodilation resulting from phosphodiesterase inhibition and likely adenosine antagonism. Dose-related acute toxicities are a particular problem with these types of compounds. As a result, routine serum concentration must be monitored in order to account for the toxicity and narrow therapeutic range arising from individual differences in metabolic clearance. Side effects include tachycardia, tachyarrhythmias, nausea and vomiting, central nervous system stimulation, headache, seizures, hematemesis, hyperglycemia and hypokalemia. Short-acting  $\beta_2$  agonists include, but are not limited to, albuterol, bitolterol, pirbuterol, and terbutaline. Some of the adverse effects associated with the administration of short-acting  $\beta_2$  agonists include tachycardia, skeletal muscle tremor, hypokalemia, increased lactic acid, headache, and hyperglycemia.

Conventional methods for treating or preventing allergy have involved the use of anti-histamines or desensitization therapies. Anti-histamines and other drugs which block the effects of chemical mediators of the allergic reaction help to regulate the severity of the allergic symptoms but do not prevent the allergic reaction and have no effect on subsequent allergic responses. Desensitization therapies are performed by giving small doses of an allergen, usually by injection under the skin, in order to induce an IgG-type response against the allergen. The presence of IgG antibody helps to neutralize the production of mediators resulting from the induction of IgE antibodies, it is believed. Initially, the subject is treated with a very low dose of the allergen to avoid inducing a severe reaction and the dose is slowly increased. This type of therapy is dangerous because the subject is actually administered the compounds which cause the allergic response and severe allergic reactions can result.

Allergy medicaments include, but are not limited to, anti-histamines, steroids, and prostaglandin inducers. Anti-histamines are compounds which counteract histamine released by mast cells or basophils. These compounds are well known in the art and commonly used

- 87 -

for the treatment of allergy. Anti-histamines include, but are not limited to, astemizole, azelastine, betastastine, buclizine, ceterizine, cetirizine analogues, CS 560, desloratadine, ebastine, epinastine, fexofenadine, HSR 609, levocabastine, loratidine, mizolastine, norastemizole, terfenadine, and tranilast.

5 Prostaglandin inducers are compounds which induce prostaglandin activity. Prostaglandins function by regulating smooth muscle relaxation. Prostaglandin inducers include, but are not limited to, S-5751.

The asthma/allergy medicaments also include steroids and immunomodulators. The steroids include, but are not limited to, beclomethasone, fluticasone, triamcinolone,  
10 budesonide, corticosteroids and budesonide.

Corticosteroids include, but are not limited to, beclomethasone dipropionate, budesonide, flunisolide, fluticasone propionate, and triamcinolone acetonide. Although dexamethasone is a corticosteroid having anti-inflammatory action, it is not regularly used for the treatment of asthma/allergy in an inhaled form because it is highly absorbed and it has  
15 long-term suppressive side effects at an effective dose. Dexamethasone, however, can be used according to the invention for the treating of asthma/allergy because when administered in combination with nucleic acids of the invention it can be administered at a low dose to reduce the side effects. Some of the side effects associated with corticosteroid include cough, dysphonia, oral thrush (candidiasis), and in higher doses, systemic effects, such as adrenal  
20 suppression, osteoporosis, growth suppression, skin thinning and easy bruising. Barnes & Peterson (1993) *Am Rev Respir Dis* 148:S1-S26; and Kamada AK et al. (1996) *Am J Respir Crit Care Med* 153:1739-48.

Systemic corticosteroids include, but are not limited to, methylprednisolone, prednisolone and prednisone. Corticosteroids are associated with reversible abnormalities in  
25 glucose metabolism, increased appetite, fluid retention, weight gain, mood alteration, hypertension, peptic ulcer, and aseptic necrosis of bone. These compounds are useful for short-term (3-10 days) prevention of the inflammatory reaction in inadequately controlled persistent asthma. They also function in a long-term prevention of symptoms in severe persistent asthma to suppress and control and actually reverse inflammation. Some side  
30 effects associated with longer term use include adrenal axis suppression, growth suppression, dermal thinning, hypertension, diabetes, Cushing's syndrome, cataracts, muscle weakness, and in rare instances, impaired immune function. It is recommended that these types of

- 88 -

compounds be used at their lowest effective dose (guidelines for the diagnosis and management of asthma; expert panel report to; NIH Publication No. 97-4051; July 1997).

The immunomodulators include, but are not limited to, the group consisting of anti-inflammatory agents, leukotriene antagonists, IL-4 muteins, soluble IL-4 receptors, immunosuppressants (such as tolerizing peptide vaccine), anti-IL-4 antibodies, IL-4 antagonists, anti-IL-5 antibodies, soluble IL-13 receptor-Fc fusion proteins, anti-IL-9 antibodies, CCR3 antagonists, CCR5 antagonists, VLA-4 inhibitors, and downregulators of IgE.

Leukotriene modifiers are often used for long-term control and prevention of symptoms in mild persistent asthma. Leukotriene modifiers function as leukotriene receptor antagonists by selectively competing for LTD-4 and LTE-4 receptors. These compounds include, but are not limited to, zafirlukast tablets and zileuton tablets. Zileuton tablets function as 5-lipoxygenase inhibitors. These drugs have been associated with the elevation of liver enzymes and some cases of reversible hepatitis and hyperbilirubinemia. Leukotrienes are biochemical mediators that are released from mast cells, eosinophils, and basophils that cause contraction of airway smooth muscle and increase vascular permeability, mucous secretions and activate inflammatory cells in the airways of patients with asthma.

Other immunomodulators include neuropeptides that have been shown to have immunomodulating properties. Functional studies have shown that substance P, for instance, can influence lymphocyte function by specific receptor-mediated mechanisms. Substance P also has been shown to modulate distinct immediate hypersensitivity responses by stimulating the generation of arachidonic acid-derived mediators from mucosal mast cells. McGillies J et al. (1987) *Fed Proc* 46:196-9 (1987). Substance P is a neuropeptide first identified in 1931. Von Euler and Gaddum *J Physiol (London)* 72:74-87 (1931). Its amino acid sequence was reported by Chang et al. in 1971. Chang MM et al. (1971) *Nature New Biol* 232:86-87. The immunoregulatory activity of fragments of substance P has been studied by Siemion IZ et al. (1990) *Molec Immunol* 27:887-890 (1990).

Another class of compounds is the down-regulators of IgE. These compounds include peptides or other molecules with the ability to bind to the IgE receptor and thereby prevent binding of antigen-specific IgE. Another type of downregulator of IgE is a monoclonal antibody directed against the IgE receptor-binding region of the human IgE molecule. Thus, one type of downregulator of IgE is an anti-IgE antibody or antibody fragment. Anti-IgE is

- 89 -

being developed by Genentech. One of skill in the art could prepare functionally active antibody fragments of binding peptides which have the same function. Other types of IgE downregulators are polypeptides capable of blocking the binding of the IgE antibody to the Fc receptors on the cell surfaces and displacing IgE from binding sites upon which IgE is already bound.

One problem associated with downregulators of IgE is that many molecules do not have a binding strength to the receptor corresponding to the very strong interaction between the native IgE molecule and its receptor. The molecules having this strength tend to bind irreversibly to the receptor. However, such substances are relatively toxic since they can bind covalently and block other structurally similar molecules in the body. Of interest in this context is that the  $\alpha$  chain of the IgE receptor belongs to a larger gene family where, e.g., several of the different IgG Fc receptors are contained. These receptors are absolutely essential for the defense of the body against, e.g., bacterial infections. Molecules activated for covalent binding are, furthermore, often relatively unstable and therefore they probably have to be administered several times a day and then in relatively high concentrations in order to make it possible to block completely the continuously renewing pool of IgE receptors on mast cells and basophilic leukocytes.

Chromolyn sodium and nedocromil are used as long-term control medications for preventing primarily asthma symptoms arising from exercise or allergic symptoms arising from allergens. These compounds are believed to block early and late reactions to allergens by interfering with chloride channel function. They also stabilize mast cell membranes and inhibit activation and release of mediators from eosinophils and epithelial cells. A four to six week period of administration is generally required to achieve a maximum benefit.

Anticholinergics are generally used for the relief of acute bronchospasm. These compounds are believed to function by competitive inhibition of muscarinic cholinergic receptors. Anticholinergics include, but are not limited to, ipratropium bromide. These compounds reverse only cholinergically-mediated bronchospasm and do not modify any reaction to antigen. Side effects include drying of the mouth and respiratory secretions, increased wheezing in some individuals, and blurred vision if sprayed in the eyes.

In addition to standard asthma/allergy medicaments, other methods for treating asthma/allergy have been used either alone or in combination with established medicaments. One preferred, but frequently impossible, method of relieving allergies is allergen or initiator

- 90 -

avoidance. Another method currently used for treating allergic disease involves the injection of increasing doses of allergen to induce tolerance to the allergen and to prevent further allergic reactions.

Allergen injection therapy (allergen immunotherapy) is known to reduce the severity of allergic rhinitis. This treatment has been theorized to involve the production of a different form of antibody, a protective antibody which is termed a "blocking antibody". Cooke RA et al. (1935) Serologic Evidence of Immunity with Coexisting Sensitization in a Type of Human Allergy, *Exp Med* 62:733. Other attempts to treat allergy involve modifying the allergen chemically so that its ability to cause an immune response in the patient is unchanged, while its ability to cause an allergic reaction is substantially altered. These methods, however, can take several years to be effective and are associated with the risk of side effects such as anaphylactic shock.

The compositions and methods of the invention can be used to modulate an immune response. The ability to modulate an immune response allows for the prevention and/or treatment of particular disorders that can be affected via immune system modulation.

Treatment after a disorder has started aims to reduce, ameliorate, or altogether eliminate the disorder, and/or its associated symptoms, or prevent it from becoming worse. Treatment of subjects before a disorder has started (i.e., prophylactic treatment) aims to reduce the risk of developing the disorder. As used herein, the term "prevent" refers to the prophylactic treatment of patients who are at risk of developing a disorder (resulting in a decrease in the probability that the subject will develop the disorder), and to the inhibition of further development of an already established disorder.

Different doses may be necessary for treatment of a subject, depending on activity of the compound, manner of administration, purpose of the immunization (i.e., prophylactic or therapeutic), nature and severity of the disorder, age and body weight of the subject. The administration of a given dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units. Multiple administration of doses at specific intervals of weeks or months apart is usual for boosting antigen-specific immune responses.

Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and preferred mode of administration, an effective

- 91 -

prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to treat the particular subject. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular therapeutic agent being administered (e.g., in the case  
5 of an immunostimulatory nucleic acid, the type of nucleic acid, i.e., a CpG nucleic acid, the number of unmethylated CpG motifs or their location in the nucleic acid, the degree of modification of the backbone to the oligonucleotide, etc.), the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular nucleic acid and/or other therapeutic agent without  
10 necessitating undue experimentation.

Subject doses of the compounds described herein typically range from about 0.1  $\mu$ g to 10,000 mg, more typically from about 1  $\mu$ g/day to 8000 mg, and most typically from about 10  $\mu$ g to 100  $\mu$ g. Stated in terms of subject body weight, typical dosages range from about 0.1  $\mu$ g to 20 mg/kg/day, more typically from about 1 to 10 mg/kg/day, and most typically  
15 from about 1 to 5 mg/kg/day.

The pharmaceutical compositions containing nucleic acids and/or other compounds can be administered by any suitable route for administering medications. A variety of administration routes are available. The particular mode selected will depend, of course, upon the particular agent or agents selected, the particular condition being treated, and the  
20 dosage required for therapeutic efficacy. The methods of this invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of an immune response without causing clinically unacceptable adverse effects. Preferred modes of administration are discussed herein. For use in therapy, an effective amount of the nucleic acid and/or other therapeutic agent can be  
25 administered to a subject by any mode that delivers the agent to the desired surface, e.g., mucosal, systemic.

Administering the pharmaceutical composition of the present invention may be accomplished by any means known to the skilled artisan. Routes of administration include but are not limited to oral, parenteral, intravenous, intramuscular, intranasal, sublingual,  
30 intratracheal, inhalation, subcutaneous, ocular, vaginal, and rectal. For the treatment or prevention of asthma or allergy, such compounds are preferably inhaled, ingested or administered by systemic routes. Systemic routes include oral and parenteral. Inhaled

- 92 -

medications are preferred in some embodiments because of the direct delivery to the lung, the site of inflammation, primarily in asthmatic patients. Several types of devices are regularly used for administration by inhalation. These types of devices include metered dose inhalers (MDI), breath-actuated MDI, dry powder inhaler (DPI), spacer/holding chambers in  
5 combination with MDI, and nebulizers.

The therapeutic agents of the invention may be delivered to a particular tissue, cell type, or to the immune system, or both, with the aid of a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the compositions to the target cells. The vector generally transports the immunostimulatory nucleic acid, antibody, antigen,  
10 and/or disorder-specific medicament to the target cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector.

In general, the vectors useful in the invention are divided into two classes: biological vectors and chemical/physical vectors. Biological vectors and chemical/physical vectors are useful in the delivery and/or uptake of therapeutic agents of the invention.

15 Most biological vectors are used for delivery of nucleic acids and this would be most appropriate in the delivery of therapeutic agents that are or that include immunostimulatory nucleic acids.

In addition to the biological vectors discussed herein, chemical/physical vectors may be used to deliver therapeutic agents including immunostimulatory nucleic acids, antibodies,  
20 antigens, and disorder-specific medicaments. As used herein, a "chemical/physical vector" refers to a natural or synthetic molecule, other than those derived from bacteriological or viral sources, capable of delivering the nucleic acid and/or other medicament.

A preferred chemical/physical vector of the invention is a colloidal dispersion system. Colloidal dispersion systems include lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system of the invention is a  
25 liposome. Liposomes are artificial membrane vessels which are useful as a delivery vector *in vivo* or *in vitro*. It has been shown that large unilamellar vesicles (LUVs), which range in size from 0.2 - 4.0  $\mu\text{m}$  can encapsulate large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically  
30 active form. Fraley et al. (1981) *Trends Biochem Sci* 6:77.

Liposomes may be targeted to a particular tissue by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein. Ligands which

- 93 -

may be useful for targeting a liposome to an immune cell include, but are not limited to: intact or fragments of molecules which interact with immune cell specific receptors and molecules, such as antibodies, which interact with the cell surface markers of immune cells. Such ligands may easily be identified by binding assays well known to those of skill in the art. In still other embodiments, the liposome may be targeted to the cancer by coupling it to a one of the immunotherapeutic antibodies discussed earlier. Additionally, the vector may be coupled to a nuclear targeting peptide, which will direct the vector to the nucleus of the host cell.

Lipid formulations for transfection are commercially available from QIAGEN, for example, as EFFECTENE<sup>TM</sup> (a non-liposomal lipid with a special DNA condensing enhancer) and SUPERFECT<sup>TM</sup> (a novel acting dendrimeric technology).

Liposomes are commercially available from Gibco BRL, for example, as LIPOFECTIN<sup>TM</sup> and LIPOFECTACE<sup>TM</sup>, which are formed of cationic lipids such as N-[1-(2, 3 dioleoyloxy)-propyl]-N, N, N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications. Liposomes also have been reviewed by Gregoriadis G (1985) *Trends Biotechnol* 3:235-241.

In one embodiment, the vehicle is a biocompatible microparticle or implant that is suitable for implantation or administration to the mammalian recipient. Exemplary bioerodible implants that are useful in accordance with this method are described in PCT International application no. PCT/US/03307 (Publication No. WO95/24929, entitled "Polymeric Gene Delivery System". PCT/US/0307 describes a biocompatible, preferably biodegradable polymeric matrix for containing an exogenous gene under the control of an appropriate promoter. The polymeric matrix can be used to achieve sustained release of the therapeutic agent in the subject.

The polymeric matrix preferably is in the form of a microparticle such as a microsphere (wherein the nucleic acid and/or the other therapeutic agent is dispersed throughout a solid polymeric matrix) or a microcapsule (wherein the nucleic acid and/or the other therapeutic agent is stored in the core of a polymeric shell). Other forms of the polymeric matrix for containing the therapeutic agent include films, coatings, gels, implants, and stents. The size and composition of the polymeric matrix device is selected to result in favorable release kinetics in the tissue into which the matrix is introduced. The size of the

- 94 -

polymeric matrix further is selected according to the method of delivery which is to be used, typically injection into a tissue or administration of a suspension by aerosol into the nasal and/or pulmonary areas. Preferably when an aerosol route is used the polymeric matrix and the nucleic acid and/or the other therapeutic agent are encompassed in a surfactant vehicle.

5 The polymeric matrix composition can be selected to have both favorable degradation rates and also to be formed of a material which is bioadhesive, to further increase the effectiveness of transfer when the matrix is administered to a nasal and/or pulmonary surface that has sustained an injury. The matrix composition also can be selected not to degrade, but rather, to release by diffusion over an extended period of time. In some preferred embodiments, the  
10 nucleic acid are administered to the subject via an implant while the other therapeutic agent is administered acutely. Biocompatible microspheres that are suitable for delivery, such as oral or mucosal delivery, are disclosed in Chickering et al. (1996) *Biotech Bioeng* 52:96-101 and Mathiowitz E et al. (1997) *Nature* 386:410-414 and PCT Pat. Application WO97/03702.

Both non-biodegradable and biodegradable polymeric matrices can be used to deliver  
15 the nucleic acid and/or the other therapeutic agent to the subject. Biodegradable matrices are preferred. Such polymers may be natural or synthetic polymers. The polymer is selected based on the period of time over which release is desired, generally in the order of a few hours to a year or longer. Typically, release over a period ranging from between a few hours and three to twelve months is most desirable, particularly for the nucleic acid agents. The  
20 polymer optionally is in the form of a hydrogel that can absorb up to about 90% of its weight in water and further, optionally is cross-linked with multi-valent ions or other polymers.

Bioadhesive polymers of particular interest include bioerodible hydrogels described by H.S. Sawhney, C.P. Pathak and J.A. Hubell in *Macromolecules*, (1993) 26:581-587, the teachings of which are incorporated herein. These include polyhyaluronic acids, casein,  
25 gelatin, gluten, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

30 If the therapeutic agent is a nucleic acid, the use of compaction agents may also be desirable. Compaction agents also can be used alone, or in combination with, a biological or chemical/physical vector. A "compaction agent", as used herein, refers to an agent, such as a

- 95 -

histone, that neutralizes the negative charges on the nucleic acid and thereby permits compaction of the nucleic acid into a fine granule. Compaction of the nucleic acid facilitates the uptake of the nucleic acid by the target cell. The compaction agents can be used alone, i.e., to deliver a nucleic acid in a form that is more efficiently taken up by the cell or, more preferably, in combination with one or more of the above-described vectors.

Other exemplary compositions that can be used to facilitate uptake of a nucleic acid include calcium phosphate and other chemical mediators of intracellular transport, microinjection compositions, electroporation and homologous recombination compositions (e.g., for integrating a nucleic acid into a preselected location within the target cell chromosome).

The compounds may be administered alone (e.g., in saline or buffer) or using any delivery vectors known in the art. For instance the following delivery vehicles have been described: cochleates (Gould-Fogerite et al., 1994, 1996); Emulsomes (Vancott et al., 1998, Lowell et al., 1997); ISCOMs (Mowat et al., 1993, Carlsson et al., 1991, Hu et al., 1998, Morein et al., 1999); liposomes (Childers et al., 1999, Michalek et al., 1989, 1992, de Haan 1995a, 1995b); live bacterial vectors (e.g., *Salmonella*, *Escherichia coli*, *Bacillus calmette-guerin*, *Shigella*, *Lactobacillus*) (Hone et al., 1996, Pouwels et al., 1998, Chatfield et al., 1993, Stover et al., 1991, Nugent et al., 1998); live viral vectors (e.g., Vaccinia, adenovirus, Herpes Simplex) (Gallichan et al., 1993, 1995, Moss et al., 1996, Nugent et al., 1998, Flexner et al., 1988, Morrow et al., 1999); microspheres (Gupta et al., 1998, Jones et al., 1996, Maloy et al., 1994, Moore et al., 1995, O'Hagan et al., 1994, Eldridge et al., 1989); nucleic acid vaccines (Fynan et al., 1993, Kuklin et al., 1997, Sasaki et al., 1998, Okada et al., 1997, Ishii et al., 1997); polymers (e.g. carboxymethylcellulose, chitosan) (Hamajima et al., 1998, Jabbal-Gill et al., 1998); polymer rings (Wyatt et al., 1998); proteosomes (Vancott et al., 1998, Lowell et al., 1988, 1996, 1997); sodium fluoride (Hashi et al., 1998); transgenic plants (Tacket et al., 1998, Mason et al., 1998, Haq et al., 1995); virosomes (Gluck et al., 1992, Mengiardi et al., 1995, Cryz et al., 1998); and, virus-like particles (Jiang et al., 1999, Leibl et al., 1998).

The formulations of the invention are administered in pharmaceutically acceptable solutions, which may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients.

- 96 -

The term pharmaceutically-acceptable carrier means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a human or other vertebrate animal. The term carrier denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the compounds of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

For oral administration, the compounds (i.e., nucleic acids, antigens, antibodies, and other therapeutic agents) can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated.

Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers for neutralizing internal acid conditions or may be administered without any carriers.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol

- 97 -

or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion.

Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

- 98 -

Alternatively, the active compounds may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as  
5 cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long-acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly  
10 soluble salt.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

15 Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules,  
20 suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of  
25 methods for drug delivery, see Langer R (1990) *Science* 249:1527-1533, which is incorporated herein by reference.

The nucleic acids and optionally other therapeutics and/or antigens may be administered *per se* (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically  
30 acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene

- 99 -

sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Suitable buffering agents include: acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the compounds into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the compounds into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product. Liquid dose units are vials or ampoules. Solid dose units are tablets, capsules and suppositories.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the compounds, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-, di-, and tri-glycerides; hydrogel release systems; silastic systems; peptide-based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which an agent of the invention is contained in a form within a matrix such as those described in U.S. Pat. Nos. 4,452,775, 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Pat. Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

- 100 -

The invention also provides efficient methods of identifying immunostimulatory compounds and optimizing the compounds and agents so identified. Generally, the screening methods involve assaying for compounds which inhibit or enhance signaling through a particular TLR. The methods employ a TLR, a suitable reference ligand for the TLR, and a candidate immunostimulatory compound. The selected TLR is contacted with a suitable reference compound (TLR ligand) and a TLR-mediated reference signal is measured. The selected TLR is also contacted with a candidate immunostimulatory compound and a TLR-mediated test signal is measured. The test signal and the reference signal are then compared. A favorable candidate immunostimulatory compound may subsequently be used as a reference compound in the assay. Such methods are adaptable to automated, high throughput screening of candidate compounds. Examples of such high throughput screening methods are described in U.S. Pat. Nos. 6,103,479; 6,051,380; 6,051,373; 5,998,152; 5,876,946; 5,708,158; 5,443,791; 5,429,921; and 5,143,854.

As used herein "TLR signaling" refers to an ability of a TLR polypeptide to activate the Toll/IL-1R (TIR) signaling pathway, also referred to herein as the TLR signal transduction pathway. Changes in TLR activity can be measured by assays designed to measure expression of genes under control of  $\kappa$ B-sensitive promoters and enhancers. Such genes can be naturally occurring genes or they can be genes artificially introduced into a cell. Naturally occurring reporter genes include the genes encoding IL-1 $\beta$ , IL-6, IL-8, the p40 subunit of interleukin 12 (IL-12 p40), and the costimulatory molecules CD80 and CD86. Other genes can be placed under the control of such regulatory elements and thus serve to report the level of TLR signaling.

The assay mixture comprises a candidate immunostimulatory compound. Typically, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a different response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration of agent or at a concentration of agent below the limits of assay detection. Candidate immunostimulatory compounds may encompass numerous chemical classes, although typically they are organic compounds. In some embodiments, the candidate immunostimulatory compounds are small RNAs or small organic compounds, i.e., organic compounds having a molecular weight of more than 50 yet less than about 2500 Daltons. Polymeric candidate immunostimulatory compounds can have higher molecular weights, e.g., oligonucleotides in the range of about 2500 to about 12,500.

- 101 -

Candidate immunostimulatory compounds also may be biomolecules such as nucleic acids, peptides, saccharides, fatty acids, sterols, isoprenoids, purines, pyrimidines, derivatives or structural analogs of the above, or combinations thereof and the like. Where the candidate immunostimulatory compound is a nucleic acid, the candidate immunostimulatory compound typically is a DNA or RNA molecule, although modified nucleic acids having non-natural bonds or subunits are also contemplated.

Candidate immunostimulatory compounds may be obtained from a wide variety of sources, including libraries of natural, synthetic, or semisynthetic compounds, or any combination thereof. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides, synthetic organic combinatorial libraries, phage display libraries of random peptides, and the like. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural and synthetically produced libraries and compounds can be readily modified through conventional chemical, physical, and biochemical means. Further, known pharmacological agents may be subjected to directed or random chemical modifications such as acylation, alkylation, esterification, amidification, etc., to produce structural analogs of the candidate immunostimulatory compounds.

A variety of other reagents also can be included in the mixture. These include reagents such as salts, buffers, neutral proteins (e.g., albumin), detergents, etc., which may be used to facilitate optimal protein-protein and/or protein-nucleic acid binding. Such a reagent may also reduce non-specific or background interactions of the reaction components. Other reagents that improve the efficiency of the assay such as protease inhibitors, nuclease inhibitors, antimicrobial agents, and the like may also be used.

The order of addition of components, incubation temperature, time of incubation, and other parameters of the assay may be readily determined. Such experimentation merely involves optimization of the assay parameters, not the fundamental composition of the assay. Incubation temperatures typically are between 4°C and 40°C, more typically about 37°C. Incubation times preferably are minimized to facilitate rapid, high throughput screening, and typically are between 1 minute and 10 hours.

After incubation, the level of TLR signaling is detected by any convenient method available to the user. For cell-free binding type assays, a separation step is often used to

- 102 -

separate bound from unbound components. The separation step may be accomplished in a variety of ways. For example, separation can be accomplished in solution, or, conveniently, at least one of the components is immobilized on a solid substrate, from which the unbound components may be easily separated. The solid substrate can be made of a wide variety of materials and in a wide variety of shapes, e.g., microtiter plate, microbead, dipstick, resin particle, etc. The substrate preferably is chosen to maximize signal-to-noise ratios, primarily to minimize background binding, as well as for ease of separation and cost.

Separation may be effected, for example, by removing a bead or dipstick from a reservoir, emptying or diluting a reservoir such as a microtiter plate well, rinsing a bead, particle, chromatographic column or filter with a wash solution or solvent. The separation step preferably includes multiple rinses or washes. For example, when the solid substrate is a microtiter plate, the wells may be washed several times with a washing solution, which typically includes those components of the incubation mixture that do not participate in specific bindings such as salts, buffer, detergent, non-specific protein, etc. Where the solid substrate is a magnetic bead, the beads may be washed one or more times with a washing solution and isolated using a magnet.

Detection may be effected in any convenient way for cell-based assays such as measurement of an induced polypeptide within, on the surface of, or secreted by the cell. Examples of detection methods useful in cell-based assays include fluorescence-activated cell sorting (FACS) analysis, bioluminescence, fluorescence, enzyme-linked immunosorbent assay (ELISA), reverse transcriptase-polymerase chain reaction (RT-PCR), and the like. Examples of detection methods useful in cell-free assays include bioluminescence, fluorescence, ELISA, RT-PCR, and the like.

## Examples

### Example 1. Responsiveness of Human PBMC to G,U-Containing Oligoribonucleotides.

Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors, plated at  $3 \times 10^5$  cells/well, stimulated in vitro with various test and control immunostimulatory agents for 16 hours, and then analyzed by enzyme-linked immunosorbent assay (ELISA) using matched antibody pairs from BD-Pharmingen for secreted cytokines IL-12 p40 and TNF- $\alpha$ , performed according to the manufacturer's protocol. Also included were certain negative controls, including medium alone and DOTAP (10  $\mu$ g/200 $\mu$ l culture well;

- 103 -

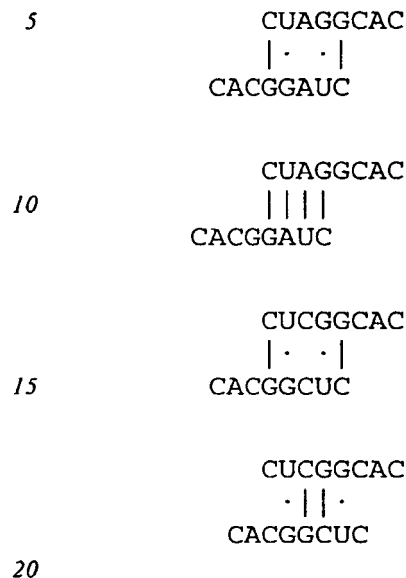
“Liposomes”) alone. The control immunostimulatory agents included the imidazoquinolone R-848 (2 µg/ml), lipopolysaccharide (LPS; 1 µg/ml), Pam3Cys (5 µg/ml), poly IC (50 µg/ml), and CpG DNA (50 µg/ml). These are reported ligands for TLR7, TLR4, TLR2, TLR3, and TLR9, respectively. Test immunostimulatory agents included the following RNA molecules, each at 50 µg/ml, with and without DOTAP (10 µg total “with Liposomes” and “without Liposomes”, respectively): GUGUUUAC alone; GUAGGCAC alone; GUGUUUAC in combination with GUAGGCAC; GUAGGA; GAAGGCAC; CUAGGCAC; CUCGGCAC; and CCCCCCCC. These RNA oligonucleotides each contained a phosphorothioate linkage between the penultimate and 3' terminal nucleoside.

**FIG. 1** depicts the responsiveness of human PBMC to the test and control agents listed above, as measured by secreted amounts of IL-12 p40 (pg/ml). As can be seen in FIG. 1, PBMCs were responsive to R-848, LPS, Pam3Cys, and poly IC, while they were unresponsive to DOTAP alone. Significantly, human PBMC secreted large amounts of IL-12 p40 (10-20 ng/ml) in response to G,U-containing RNA oligonucleotides GUGUUUAC alone; GUAGGCAC alone; GUGUUUAC in combination with GUAGGCAC; CUAGGCAC; and CUCGGCAC, each in combination with DOTAP. Also significantly, human PBMC did not secrete significant amounts of IL-12 p40 in response to G,U-free RNA oligonucleotides GAAGGCAC and CCCCCCCC. The immunostimulatory effect of the G,U-containing RNA molecules appeared to be greatly enhanced by the inclusion of DOTAP. In this experiment, the G,U-containing 6-mer RNA GUAGGA appeared to exert little, if any immunostimulatory effect either with or without DOTAP.

**FIG. 2** depicts the responsiveness of human PBMC to the test and control agents listed above, as measured by secreted amounts of TNF-α. A similar pattern of results was observed as in FIG. 1, i.e., human PBMC secreted large amounts of TNF-α (40-100 ng/ml) in response to G,U-containing RNA oligonucleotides GUGUUUAC alone; GUAGGCAC alone; GUGUUUAC in combination with GUAGGCAC; CUAGGCAC; and CUCGGCAC, each in combination with DOTAP. Also similar to the results in FIG. 1, human PBMC did not secrete significant amounts of TNF-α in response to G,U-free RNA oligonucleotides GAAGGCAC and CCCCCCCC, or in response to the G,U-containing 6-mer RNA GUAGGA. The immunostimulatory effect of the G,U-containing RNA molecules appeared to be greatly enhanced by the inclusion of DOTAP.

- 104 -

It will be appreciated in this example that the following partial self-complementarity basepairing is possible, where G-U wobble basepairs are shown joined with a dot and G-C and A-U basepairs are shown joined by a line:



Example 2. Dose-Response Behavior of Human PBMC to G,U-Containing Oligoribonucleotides.

The experiments described in the preceding example were repeated with varied concentrations of RNA oligonucleotides in order to assess the dose-response behavior of human PBMCs to G,U-containing RNA oligonucleotides of the invention. A total of 10, 3 or 1 µg RNA was added to 10 µg DOTAP and then added to the 200µl culture wells. After 16 hours IL-12 p40 and TNF-α ELISAs were performed as described in Example 1.

**FIG. 3** depicts the dose-response of human PBMC to the various RNAs as measured by secreted amounts of IL-12 p40 (ng/ml). As can be seen from FIG. 3, human PBMC secreted increasing amounts of IL-12 p40 in response to increasing amounts of G,U-containing RNA oligomers GUGUUUAC; GUAGGCAC; CUAGGCAC; and CUCGGCAC, each in combination with DOTAP. Conversely, FIG. 3 also shows that human PBMC appeared not to secrete IL-12 p40 in response to any of the tested amounts of G,U-free RNA oligomers GAAGGCAC or CCCCCCCC.

Corresponding dose-response of human PBMC to the various RNAs was measured by secreted amounts of TNF-α. A similar pattern of results was observed as in FIG. 3, i.e.,

- 105 -

human PBMC secreted increasing amounts of TNF- $\alpha$  in response to increasing amounts G,U-containing RNA oligonucleotides GUGUUUAC; GUAGGCAC; CUAGGCAC; and CUCGGCAC, each in combination with DOTAP. Also similar to the results in FIG. 3, human PBMC did not appear to secrete significant amounts of TNF- $\alpha$  in response to any of the tested amounts of G,U-free RNA oligonucleotides GAAGGCAC and CCCCCCCC.

#### Example 3. Base Sequence Sensitivity of RNA Oligomers

Point mutations were made to the RNA oligonucleotide GUAGGCAC by substituting A or C at selected positions. The various oligoribonucleotides included the following:

10 GUAGGCAC; GUAGGA; GAAGGCAC; AUAAACAC; AUAGACAC; AUAAGCAC; GUAAACAC; CUAGGCAC; CUCGGCAC; and GUGUUUAC. The oligonucleotides were titrated onto human PBMC isolated from healthy donors and plated at  $3 \times 10^5$  cells/well. A total of 10  $\mu$ g RNA was added to 10  $\mu$ g DOTAP and then added to the 200  $\mu$ l culture wells. Human TNF- $\alpha$  was measured by ELISA using matched antibody pairs from BD-Pharmingen

15 according to the manufacturer's protocol. Results are shown in FIG. 4.

#### Example 4. Effect of DOTAP on Human PBMC Response to Various Stimuli.

In order to characterize further the role of DOTAP in the immunostimulatory effects of the G,U-containing RNA oligomers observed in the previous examples, human PBMCs

20 were isolated from healthy donors, plated at  $3 \times 10^5$  cells/well, and stimulated in the presence of known TLR ligands, either with or without DOTAP ("with Liposomes" or "without Liposomes", respectively). The known TLR ligands examined were total RNA prepared from hyphae (hyphae), total RNA prepared from yeast (yeast), total RNA prepared from promyelocytic cell line HL-60 (HL60), in vitro transcribed ribosomal RNA for *E. coli* Sp6, in

25 vitro transcribed ribosomal RNA for *E. coli* T7, LPS, poly IC, Pam3Cys, and R-848. Medium alone and DOTAP alone were used as negative controls. The panel of RNAs from the previous examples, again at 10  $\mu$ g/ml and without DOTAP, was also included.

Total RNA was isolated from the human promyelocytic cell line HL-60 using Trizol (Sigma). Prior to isolation, cells were treated for 4 hours with 500  $\mu$ M hydrogen peroxide

30 ( $H_2O_2$ ), which induces apoptosis in this cell line (HL60 500). Untreated cells served as control (HL60 0).

- 106 -

*Candida albicans* RNA was isolated from yeast or hyphae (induced by 4h incubation with 10% fetal calf serum). Cells from a 100 ml culture were pelleted, washed and resuspended in 10 ml of Tris/EDTA buffer (10mM, 1mM). RNA was isolated by extraction with hot acidic phenol according to methods described in Ausubel FM et al., eds., Current  
5 Protocols in Molecular Biology, John Wiley & Sons, New York.

The genomic fragment of *E. coli* 16S RNA was amplified with the primers 5'-ATTGAAGAGTTTGATCATGGCTCAGATTGAACG-3' (SEQ ID NO:5) and 5'-TAAGGAGGTGATCCAACCGCAGGTTCC-3' (SEQ ID NO:6) from genomic *E. coli* DNA and cloned into the pGEM T easy vector. In vitro transcription was performed using  
10 T7 or Sp6 RNA polymerase. Transcribed RNA was further purified by chloroform/phenol extraction, precipitated, and used at 10 µg.

Following 16 hour incubation, ELISAs were performed as before to assess secretion of IL-12 p40 and TNF-α. Representative results are shown in FIG. 5.

**FIG. 5** depicts the effect of DOTAP on the amount of IL-12 p40 secreted by human  
15 PBMC following incubation with and without DOTAP. As can be seen from the figure, the following stimuli appeared to exert greater immunostimulatory effect in the presence of DOTAP than in its absence: hyphae, yeast, *E. coli* Sp6, and *E. coli* T7. The following stimuli appeared to exert reduced immunostimulatory effect in the presence of DOTAP than in its absence: LPS, poly IC. The following stimuli appeared to exert about the same  
20 immunostimulatory effect in the presence or absence of DOTAP: HL60, Pam3Cys and R-848.

Example 5. Immunostimulatory Effect of G,U-Containing RNA Oligomers Is Species- and MyD88-Dependent.

The following murine cells were isolated and incubated with various RNAs and other  
25 known TLR ligands in order to assess species-, cell type-, and signaling pathway- specificity: wild type macrophages in the presence of IFN-γ; MyD88-deficient macrophages in the presence of IFN-γ; J774 (mouse macrophage cell line); and RAW 264.7 (mouse macrophage cell line, e.g., ATCC TIB-71). Murine bone macrophages were generated from wild type or MyD88-deficient C57BL/6 mice by culturing bone marrow cells with 50 ng/ml M-CSF for 5  
30 days. Cells were seeded at 25,000 cells/well and treated with 20 ng/ml IFN-γ for 16 hours. The murine macrophage cell lines RAW and J774 were seeded at 10,000 cells/well.

- 107 -

The following test and control agents were examined: R-848 (2 µg/ml), ODN 1668 (CpG DNA; 5'-TCCATGACGTTTCCTGATGCT-3'; SEQ ID NO:7); LPS (1 µg/ml); poly IC (50 µg/ml); Pam3Cys (5 µg/ml); Ionomycin/TPA; the following RNA molecules, each with ("+" Lipo") and without DOTAP (10 µg/200 µl culture well): GUGUUUAC alone (RNA1);  
5 GUAGGCAC alone (RNA2); GUGUUUAC in combination with GUAGGCAC (RNA1/2); UCCGCAAUGGACGAAAGUCUGACGGA (RNA6; SEQ ID NO:8); GAGAUGGGUGCGAGAGCGUCAGUAUU (RNA9; SEQ ID NO:9); and the following DNA molecules, corresponding to RNA1, RNA2, and RNA1/2: GTGTTTAC alone (DNA1); GTAGGCAC alone (DNA2); and GTGTTTAC in combination with GTAGGCAC  
10 (DNA1/2). These RNA and DNA oligonucleotides each contained a phosphorothioate linkage between the penultimate and 3' terminal nucleoside. RNA6 and RNA9 each contained in addition a phosphorothioate linkage between the penultimate and 5' terminal nucleoside. RNA6 corresponds to a ribosomal RNA stem loop derived from *Listeria monocytogenes*. RNA9 corresponds to a stem loop derived from human immunodeficiency  
15 virus (HIV, an RNA retrovirus). The cells were cultured for 12 hours and supernatants were harvested. Murine IL-12 p40, IL-6, and TNF-α were measured by ELISA using matched antibody pairs from BD-Pharmingen according to the manufacturer's protocol. Representative results are shown in FIG. 6.

Panel A of FIG. 6 shows that wild type murine macrophages in the presence of IFN-γ  
20 secrete significant amounts of IL-12 p40 in response to R-848; ODN 1668 (CpG DNA); LPS; poly IC; Pam3Cys; and G,U-containing RNA oligomers GUGUUUAC in combination with GUAGGCAC (with DOTAP). In contrast, Panel B of FIG. 6 shows that MyD88-deficient murine macrophages in the presence of IFN-γ secrete little or no IL-12 p40 in response to any of the test and control agents examined, thus demonstrating a dependence on MyD88 for  
25 immunostimulatory response to these compounds. Such a result is consistent with participation by a TLR in the immunostimulatory response to any of these compounds, including in particular the G,U-containing RNA oligonucleotides of the invention. Panels C and D of FIG. 6 show generally similar, if somewhat attenuated, response patterns of J774 and RAW 264.7 mouse macrophage cell lines as for wild type murine macrophages in the  
30 presence of IFN-γ, as shown in Panel A. Essentially similar results were found in parallel ELISAs measuring IL-6 and TNF-α.

- 108 -

In additional studies involving MyD88 wild-type cells, it was observed that addition of bafilomycin largely or completely abrogated the immunostimulatory effect of the RNA oligomers. Together with the MyD88-dependence, this observation is consistent with involvement of at least one of TLR3, TLR7, TLR8, and TLR9.

5

#### Example 6. Use of Cholesteryl Ester in Place of Cationic Lipid

In order to investigate the possibility of using cholesteryl ester-modified RNA oligomer in place of RNA oligomer plus cationic lipid, RNA oligomer GUGUGUGU was prepared with (R 1058) and without (R 1006) a 3' cholesteryl ester modification. These two RNA oligomers with and without DOTAP, were added over a range of concentrations to overnight cultures of human PBMC. Culture supernatants were harvested, and human TNF- $\alpha$ , IL-12 p40, and IFN- $\alpha$  were measured by ELISA using matched antibody pairs from BD-Pharmingen according to the manufacturer's protocol. Representative results for experiments including DOTAP are shown in Table 1.

15

Table 1. Cholesteryl Ester Modification in Place of DOTAP

ID	TNF- $\alpha$ + DOTAP		TNF- $\alpha$ - DOTAP		IFN- $\alpha$ + DOTAP		IFN- $\alpha$ - DOTAP	
	EC50 $\mu$ M	max pg/ml	EC50, $\mu$ M	max pg/ml	EC50 $\mu$ M	max pg/ml	EC50 $\mu$ M	max pg/ml
R 1006	2.8	40000	7.8	2200	4.5	5000	--	--
R 1058	0.2	75000	1.0	3000	0.5	3800	0.5	1500

The results indicate that R 1058, with the cholesteryl ester modification, is more potent than R 1006, having the same base sequence but without cholesterol, both with and without DOTAP.

20

#### Example 7. Effect of Oligomer Length.

RNA oligomers GUGUGUGU, GUGUGUG, GUGUGU, GUGUG, GUGU, GUG, and GU, with and without DOTAP, were added over a range of concentrations to overnight cultures of human PBMC. Culture supernatants were harvested, and human TNF- $\alpha$ , IL-12 p40, and IFN- $\alpha$  were measured by ELISA using matched antibody pairs from BD-Pharmingen according to the manufacturer's protocol. Representative results for experiments including DOTAP are shown in Table 2.

25

Table 2. Effect of RNA Oligomer Length

ID	SEQ	TNF- $\alpha$		IL-12 p40		IFN- $\alpha$	
		EC50, $\mu$ M	max pg/ml	EC50, $\mu$ M	max pg/ml	EC50, $\mu$ M	max pg/ml
R 1006	GUGUGUGU	2.8	40000	1.6	7000	4.5	5000
R 1048	GUGUGUG	2.2	30000	2.6	10000	4.6	2700
R 1049	GUGUGU	6.7	30000	2.1	8000	4.8	3400
R 1050	GUGUG	7.6	40000	3.9	14000	6.9	400
R 1051	GUGU	--	--	>20	14000	--	--
R 1052	GUG	--	--	>20	6000	5.5	800
R 1053	GU	--	--	>20	5000	--	--

Example 8. Effect of Stabilization of Internucleoside Linkages.

5 GUGUGUGU RNA oligomers were synthesized with specific phosphorothioate and phosphodiester linkages as shown in Table 2, where “\*” represents phosphorothioate and “\_” represents phosphodiester. RNA oligomers, with and without DOTAP, were added over a range of concentrations to overnight cultures of human PBMC. Culture supernatants were harvested, and human TNF- $\alpha$ , IL-12 p40, and IFN- $\alpha$  were measured by ELISA using

10 matched antibody pairs from BD-Pharmingen according to the manufacturer’s protocol. Representative results for experiments including DOTAP are shown in Table 3.

Table 3. Effect of Stabilization of Internucleoside Linkages

ID	SEQ	TNF- $\alpha$		IFN- $\alpha$	
		EC50, $\mu$ M	max, pg/ml	EC50, $\mu$ M	max, pg/ml
R 1006	G*U*G*U*G*U*G*U	2.8	40000	4.5	5000
R 1054	G*U_G*U*G*U*G*U	5.6	40000	6.7	3700
R 1055	G*U_G*U_G*U*G*U	>20	20000	--	--
R 1056	G*U_G*U_G*U_G*U	>20	12000	--	--
R 1057	G_U_G_U_G_U_G_U	--	--	0.1	6000

- 110 -

In like manner, an all-phosphodiester 40-mer capable of forming a stem-loop structure and having a base sequence as provided by

5'-CACACACUGCUUAAGCGCUUGCCUGCUUAAGUAGUGUGUG-3' (R 1041; SEQ ID NO:10) was synthesized and tested in overnight culture with human PBMC. This RNA oligomer was found to be very potent in its ability to induce IFN- $\alpha$ , with an EC<sub>50</sub> of <0.1  $\mu$ M and a maximum of 5000 pg/ml.

#### Example 9. DNA:RNA Conjugates.

A series of DNA:RNA conjugates, each containing the RNA sequence GUGUGUGU and a poly-dT or a poly-dG sequence, was prepared. The oligomers were as follows, where again "\*" represents phosphorothioate and "\_" represents phosphodiester:

G\*U\*G\*U\*G\*U\*G\*U\_dG\_dG\*dG\*dG\*dG\*dG (R 1060; SEQ ID NO:11)

dG\*dG\*dG\*dG\_dG\_G\*U\*G\*U\*G\*U\*G\*U (R 1061; SEQ ID NO:12)

15 G\*U\*G\*U\*G\*U\*G\*U\*dT\*dT\*dT\*dT\*dT\*dT (R 1062; SEQ ID NO:13)

dT\*dT\*dT\*dT\*dT\*dT\*G\*U\*G\*U\*G\*U\*G\*U (R 1063; SEQ ID NO:14)

Human PBMC were cultured overnight in the presence of added DNA:RNA conjugate, with and without DOTAP. Culture supernatants were harvested and human TNF- $\alpha$ , IL-6, IL-12 p40, IP-10, and IFN- $\alpha$  were measured by ELISA using matched antibody pairs from BD-Pharmingen according to the manufacturer's protocol. Representative results for experiments including DOTAP are shown in Table 4.

Table 4. Immunostimulatory DNA:RNA Conjugates

ID	TNF- $\alpha$		IL-6		IP-10	
	EC <sub>50</sub> , $\mu$ M	max pg/ml	EC <sub>50</sub> , $\mu$ M	max pg/ml	EC <sub>50</sub> , $\mu$ M	max pg/ml
R 1060	4.9	20000	--	--	--	--
R 1061	4.3	20000	>20	10000	1.1	180
R 1062	0.3	80000	0.4	28000	0.1	400
R 1063	0.3	60000	0.8	28000	0.1	250

- 111 -

Example 10. Transfer RNA.

Human PBMC were cultured overnight in the presence of various concentrations (1, 3, and 10 µg/ml) of tRNA obtained from wheat germ, bovine, yeast, and *E. coli* sources, added to the culture medium with and without DOTAP. Culture supernatants were harvested and human TNF-α and IL-12 p40 were measured by ELISA using matched antibody pairs from BD-Pharmingen according to the manufacturer's protocol. Yeast and *E. coli* tRNAs, and to a lesser extent bovine tRNA, induced TNF-α and IL-12 p40 when DOTAP was also present. In addition, *E. coli* tRNA at 3 and 10 µg/ml induced minor amounts of both cytokines even without DOTAP.

Example 11. HIV RNA.

Human PBMC were incubated overnight with either of two key G,U-rich sequences, namely 5'-GUAGUGUGUG-3' (SEQ ID NO:2) and 5'-GUCUGUUGUGUG-3' (SEQ ID NO:3), corresponding to nt 99-108 and 112-123 of HIV-1 strain BH10, respectively, each with and without DOTAP. Culture supernatants were harvested, and human IL-12 p40 and TNF-α were measured by ELISA using matched antibody pairs from BD-Pharmingen according to the manufacturer's protocol. Representative results are shown in FIG. 7. The figure shows that both of these RNA molecules, at micromolar concentrations in the presence of DOTAP, induced 50-100 ng/ml of TNF and 50-200 ng/ml of IL-12 p40.

Example 12. Responsiveness of Human PBMC to Stringent Response Factor.

When bacteria are starved they enter into a programmed response termed the stringent response. This involves the production of nucleic acid alarmones and ribosomal loss. Bacteria growing at high rates contain 70,000-80,000 ribosomes accounting for as much as 50% of their dry weight. As growth slows, unneeded ribosomes are hydrolyzed. It was hypothesized that rapidly growing cells in their early stationary phase contain large amounts of oligoribonucleotides that are released into the media when the cells enter a neutral pH environment.

FIG. 10 depicts the responsiveness of human PBMC to stringent response factor (SRF). SRF is produced by rapidly growing bacteria (in this case *Listeria monocytogenes*) in rich media until their late log phase. The bacteria were pelleted and resuspended in an equal volume of PBS for 24h. The mixture is centrifuged to remove the bacteria. The supernatant

- 112 -

is sterilized by passing it through a 0.2  $\mu$ m filter. The sterilized solution was passed through a molecular filter with a cutoff of 10 kDa. This fraction was separated on a C18 column and the eluant was tested. At a concentration of 5  $\mu$ g/ml SRF induced TNF from human PBMC. If SRF was treated with any of three RNases the activity was destroyed. The activity was  
5 not due to substances other than RNA because the RNase-treated SRF had near background stimulatory ability. This implied activity was due to RNA.

Example 13. Responsiveness of Human PBMC to Ribonucleoside Vanadyl Complexes.

During studies of SRF it was surprisingly determined that the RNase inhibitor,  
10 ribonucleoside vanadyl complexes (RVCs), could stimulate human PBMC to produce TNF (FIG. 11) and IL-6.

FIG. 11 depicts the responsiveness of human PBMC to the ribonucleoside vanadyl complexes (RVCs). It was unexpectedly discovered during testing of RNase inhibitors that RVCs were stimulatory for human PBMC. 2mM RVC induced the release of substantial  
15 TNF. Also tested was the anti-viral imidazoquinoline, resiquimod (R-848) denoted as X and used at 0.1  $\mu$ g/ml.

Example 14. Responsiveness of Human TLR7 and human TLR8 to Ribonucleosides.

The observations of Example 13 could be extended to 293 cells genetically  
20 reconstituted with TLR7 and TLR8 but not non-transfected 293 cells (FIG. 12). During analysis of individual ribonucleoside vanadyl complexes, it was unexpectedly determined that a mixture of the ribonucleosides A, U, C, and G or the single ribonucleoside G was effective in the absence of vanadate at stimulating PBMC to produce TNF and TLR7 or TLR8 to activate NF-kB (FIG. 12).

25 FIG. 12 depicts the responsiveness of human TLR7 and human TLR8 to ribonucleosides. It was determined that the response by human PBMC to RNA or RVC was mediated by TLR7 or TLR8 and further that the response could be driven by ribonucleosides only. Human 293 cells were either mock-transfected or transfected with human TLR7 or human TLR8 and monitored for responsiveness to ribonucleosides. The open reading frames  
30 of human TLR7 (hTLR7) and human TLR8 (hTLR8) were amplified by PCR from a cDNA library of human PBMC using the following primers pairs: for TLR7, 5'-CACCTCTCATGCTCTGCTCTCTTC-3' (SEQ ID NO:15) and

- 113 -

5'-GCTAGACCGTTTCCTTGAACACCTG-3' (SEQ ID NO:16); and for TLR8,  
5'-CTGCGCTGCTGCAAGTTACGGAATG-3' (SEQ ID NO:17) and  
5'-GCGCGAAATCATGACTTAACGTCAG-3' (SEQ ID NO:18). The sequence information  
for primer selection was obtained from Genbank accession numbers AF240467 and  
5 AF245703. All full-length TLR fragments were cloned into pGEM-T Easy vector (Promega,  
Mannheim, Germany), excised with NotI, cloned into the expression vector pcDNA 3.1(-)  
(Invitrogen, Karlsruhe, Germany) and sequenced. Sequences of the coding region of hTLR7  
and hTLR8 correspond to the accession numbers AF240467 (SEQ ID NO:25) and  
AF245703, respectively (SEQ ID NO:29).

10 For monitoring transient NF- $\kappa$ B activation,  $3 \times 10^6$  293 HEK cells (ATCC, VA, USA)  
were electroporated at 200 volt and 960  $\mu$ F with 1  $\mu$ g TLR expression plasmid, 20 ng NF- $\kappa$ B  
luciferase reporter-plasmid and 14  $\mu$ g of pcDNA3.1(-) plasmid as carrier in 400  $\mu$ l RPMI  
medium supplemented with 25% fetal bovine serum (FCS). Cells were seeded at  $10^5$  cells  
per well and after over night culture stimulated with R-848 (denoted in FIG. 12 as X;  
15 commercially synthesized by GLSynthesis Inc., Worcester, MA, USA), RVCs or  
ribonucleosides for a further 7 hours. Stimulated cells were lysed using reporter lysis buffer  
(Promega, Mannheim, Germany), and lysate was assayed for luciferase activity using a  
Berthold luminometer (Wildbad, Germany).

As depicted in FIG. 12, TLR7 transfectants responded to R-848, RVCs, a mixture of  
20 ribonucleosides (A, G, C, U at 0.5 mM) and the ribonucleoside guanosine. Likewise TLR8  
showed a similar response pattern.

#### Example 16. Responsiveness of TLR7 and TLR8 to Mixtures of Two Ribonucleosides.

FIG. 13 depicts the responsiveness of TLR7 and TLR8 to mixtures of two  
25 ribonucleosides. In an experiment conducted as in FIG. 11 it was determined that TLR 8  
responded best to a combination of the ribonucleosides G and U, however, TLR7 responded  
best to G alone. Additionally it can be seen that a minor response was given by a  
combination of C and U. These data show that ribonucleosides of the proper composition  
serve as ligands for TLR7 and TLR8. The nonspecific stimulus of TPA served as a control  
30 only. X denotes R-848.

#### Example 17. Human PBMC Respond to a Mixture of the Ribonucleosides G and U.

- 114 -

**FIG. 14** depicts the response of human PBMC to a mixture of the ribonucleosides G and U. It can be appreciated that the ribonucleosides G and U act synergistically to induce TNF from human PBMC. In this example the ratio of G:U of 1:10 was optimal.

5 Example 18. Human PBMC Respond to G,U-Rich Oligoribonucleotides.

**FIG. 15** depicts how human PBMC respond to RNA G,U-rich oligonucleotides. Both RNA and DNA oligonucleotides 5'-GUUGUGGUUGUGGUUGUG-3' (SEQ ID NOs:1 and 19) were tested at 30 $\mu$ M on human PBMC and TNF was monitored. Human PBMC were responsive to G,U-rich RNA oligonucleotides and not G,U-rich DNA oligonucleotides.

10

Example 19. Human PBMC Respond to Oxidized RNA.

**FIG. 16** depicts the response of human PBMC to oxidized RNA. Ribosomal 16S RNA was isolated from *E. coli* and subjected to chemical oxidation. The treatments were (mod A) 0.2 mM ascorbic acid plus 0.2 mM CuCl<sub>2</sub> for 30 min at 37°C or (mod B) 0.2 mM ascorbic acid plus 0.02 mM CuCl<sub>2</sub> for 30 min at 37°C. This treatment induces oxidation at the 8 position of guanosine and also induces strand breaks 3' of the modified guanosine. It was shown that ribosomal RNA induced TNF production from human PBMC. It was also evident that oxidation of ribosomal RNA greatly potentiates the response.

20 Example 20. Human TLR7 Responds to Oxidized Guanosine Ribonucleoside.

**FIG. 17** depicts human TLR7 and TLR8 responses to the oxidized guanosine ribonucleoside. Cells mock-transfected or transfected with human TLR 7 or human TLR8, as in Example 14, were tested for responsiveness to 7-allyl-8-oxoguanosine (loxoribine) at 1 mM. It can be clearly shown that human TLR7 is responsive to 7-allyl-8-oxoguanosine. Thus it appears that a ligand for TLR 7 is oxidized nucleic acids.

25

Example 21. Human TLR7 Responds to Other Modified Guanosine Ribonucleoside.

**FIG. 18** depicts human TLR7 responses to the other modified guanosine ribonucleoside. Cells transfected with human TLR7, as in Example 14, were tested for a dose-dependent response to 7-allyl-8-oxoguanosine (loxoribine). Additionally other modified guanosines were tested. It can be clearly shown that human TLR 7 was responsive to 7-allyl-8-oxoguanosine in a dose-dependent manor. As shown above, human TLR7 was

30

- 115 -

responsive to guanosine; however **FIG. 18** also shows that human TLR7 responded mildly to the deoxy form of guanosine as well as to 8-bromo-guanosine.

Example 22. Distribution of Human TLRs.

5           **FIG. 19** depicts the distribution of human TLR1-TLR9. Various purified human immune cells were screened by PCR for TLR1 through 9 expression. It was shown that human lymphoid CD123+ dendritic cells (DC) were strongly positive for TLR9 and TLR7 while weaker for TLR8. The converse was shown however for myeloid CD11c+ DC. This is very relevant because the two types of DC have very different functions in the immune  
10 system. Significantly, **FIG. 19** also shows that human neutrophils were strongly positive for human TLR8 while very weak for TLR9 and negative for TLR7. This is also relevant because neutrophils are very often the first cells to engage infectious pathogens and thus believed to initiate responses.

15           Example 23.

          HEK-293 cell were stably transfected with human TLR7 or human TLR8. Additionally, the cells were stably transfected with NF- $\kappa$ B-luciferase reporter construct. The cells were titrated with varying amounts of RNA oligonucleotides and cultured for 16h. Luciferase activity was measured by standard methods and normalized versus mock-  
20 stimulated transfectants. Luciferase activity measured for the mock-stimulated transfectant was set to a value of 1-fold NF- $\kappa$ B induction. Results are shown in **FIG. 20**, where old NF- $\kappa$ B induced by the stimulating RNA oligonucleotide is plotted versus the concentration of test ribonucleotide. Stimulation with GUGUGUGU is shown for human TLR8. Stimulation with GUAGUCAC is shown for human TLR7 and human TLR8.

25

**Equivalents**

          The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of  
30 the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall

- 116 -

within the scope of the appended claims. The advantages and objects of the invention are not necessarily encompassed by each embodiment of the invention.

All references, patents and patent publications that are recited in this application are incorporated in their entirety herein by reference.

5

We claim:

- 117 -

**Claims**

1. An immunostimulatory composition, comprising:  
an isolated RNA oligomer 5-40 nucleotides long having a base sequence comprising  
5 at least one guanine (G) and at least one uracil (U), and optionally  
a cationic lipid.
2. The composition of claim 1, wherein the isolated RNA oligomer is a G,U-rich RNA.
- 10 3. The composition of claim 1, wherein the base sequence comprises 5'-RURGY-3',  
wherein R represents purine, U represents uracil, G represents guanine, and Y represents  
pyrimidine.
4. The composition of claim 1, wherein the base sequence comprises 5'-GUAGU-3',  
15 wherein A represents adenine.
5. The composition of claim 1, wherein the base sequence comprises 5'-GUAGUGU-3'.
6. The composition of claim 1, wherein the base sequence comprises 5'-GUUGB-3',  
20 wherein B represents U, G, or C, wherein C represents cytosine.
7. The composition of claim 1, wherein the base sequence comprises 5'-GUGUG-3'.
8. The composition of claim 1, wherein the base sequence comprises  
25 5'-GUGUUUAC-3'.
9. The composition of claim 1, wherein the base sequence comprises  
5'-GUAGGCAC-3'.
- 30 10. The composition of claim 1, wherein the base sequence comprises  
5'-CUAGGCAC-3'.

- 118 -

11. The composition of claim 1, wherein the base sequence comprises 5'-CUCGGCAC-3'.

12. The composition of claim 1, wherein the oligomer is 5-12 nucleotides long.

5 13. The composition of claim 1, wherein the base sequence is free of CpG dinucleotide.

14. The composition of claim 1, wherein the base sequence is at least 50 percent self-complementary.

10 15. The composition of claim 1, wherein the oligomer is a plurality of oligomers.

16. The composition of claim 15, wherein the plurality of oligomers comprises an oligomer having a first base sequence and an oligomer having a second base sequence, wherein the first base sequence and the second base sequence are at least 50 percent  
15 complementary.

17. The composition of claim 15, wherein the plurality of oligomers comprises an oligomer having a base sequence comprising 5'-GUGUUUAC-3' and an oligomer having a base sequence comprising 5'-GUAGGCAC-3'.

20

18. The composition of claim 1, wherein the oligomer comprises a non-natural backbone linkage.

19. The composition of claim 18, wherein the non-natural backbone linkage is a  
25 phosphorothiate linkage.

20. The composition of claim 1, wherein the oligomer comprises a modified base selected from the group consisting of 7-deazaguanosine, 8-azaguanosine, 5-methyluracil, and pseudouracil.

30

21. The composition of claim 1, wherein the oligomer comprises a modified sugar.

- 119 -

22. The composition of claim 1, wherein the cationic lipid is N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP).
23. The composition of claim 1, further comprising an antigen.
24. The composition of claim 23, wherein the antigen is an allergen.
25. The composition of claim 23, wherein the antigen is a cancer antigen.
26. The composition of claim 23, wherein the antigen is a microbial antigen.
27. The composition of claim 1, further comprising a pharmaceutically acceptable carrier.
28. A method of activating an immune cell, comprising:  
contacting an immune cell with the composition of any one of claims 1-27 in an effective amount to induce activation of the immune cell.
29. The method of claim 28, wherein the activation of the immune cell comprises secretion of a cytokine or chemokine by the immune cell.
30. The method of claim 29, wherein the cytokine is selected from the group consisting of interleukin 6 (IL-6), interleukin 12 (IL-12), an interferon (IFN), and tumor necrosis factor (TNF).
31. The method of claim 29, wherein the chemokine is interferon-gamma-inducible protein 10 (IP-10).
32. The method of claim 28, wherein the activation of the immune cell comprises activation of a MyD88-dependent signal transduction pathway.
33. The method of claim 32, wherein the MyD88-dependent signal transduction pathway is associated with a Toll-like receptor (TLR).

- 120 -

34. The method of claim 33, wherein the TLR is TLR8.

35. The method of claim 33, wherein the TLR is TLR7.

5

36. A method of inducing an immune response in a subject, comprising:  
administering to a subject a composition of any one of claims 1-27 in an effective  
amount to induce an immune response in the subject.

10 37. The method of claim 36, wherein the subject has or is at risk of having a cancer.

38. The method of claim 36, wherein the subject has or is at risk of having an infection  
with an agent selected from the group consisting of viruses, bacteria, fungi, and parasites.

15 39. The method of claim 36, wherein the subject has or is at risk of having a viral  
infection.

40. A method of inducing an immune response in a subject, comprising:  
administering to a subject an antigen; and

20 administering to the subject a composition of any one of claims 1-22 or 27 in an  
effective amount to induce an immune response to the antigen.

41. The method of claim 40, wherein the antigen is an allergen.

25 42. The method of claim 40, wherein the antigen is a cancer antigen.

43. The method of claim 40, wherein the antigen is a microbial antigen.

44. A method of inducing an immune response in a subject, comprising:

30 isolating dendritic cells of a subject;

contacting the dendritic cells ex vivo with the composition of any one of claims 1-22  
or 27;

- 121 -

contacting the dendritic cells ex vivo with an antigen; and  
administering the contacted dendritic cells to the subject.

45. The method of claim 44, wherein the antigen is an allergen.
- 5
46. The method of claim 44, wherein the antigen is a cancer antigen.
47. The method of claim 44, wherein the antigen is a microbial antigen.
- 10 48. A composition, comprising:  
an effective amount of a ligand for Toll-like receptor 8 (TLR8) to induce TLR8  
signaling, and  
an effective amount of a ligand for a second TLR selected from the group consisting  
of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR9 and TLR10 to induce  
15 signaling by the second TLR.
49. The composition according to claim 48, wherein the second TLR is TLR3.
50. The composition according to claim 48, wherein the second TLR is TLR7.
- 20 51. The composition according to claim 48, wherein the second TLR is TLR9.
52. The composition according to claim 48, wherein the ligand for TLR8 and the ligand  
for the second TLR are linked.
- 25 53. The composition according to claim 48, further comprising a pharmaceutically  
acceptable carrier.
54. A composition, comprising:  
30 an effective amount of a ligand for TLR7 to induce TLR7 signaling, and

- 122 -

an effective amount of a ligand for a second TLR selected from the group consisting of: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR8, TLR9, and TLR10 to induce signaling by the second TLR.

- 5 55. The composition according to claim 54, wherein the second TLR is TLR3.
56. The composition according to claim 54, wherein the second TLR is TLR8.
57. The composition according to claim 54, wherein the second TLR is TLR9.
- 10 58. The composition according to claim 54, wherein the ligand for TLR7 and the ligand for the second TLR are linked.
59. The composition according to claim 54, further comprising a pharmaceutically  
15 acceptable carrier.
60. A composition, comprising:  
a DNA:RNA conjugate, wherein DNA of the conjugate comprises a CpG motif  
effective for stimulating TLR9 signaling and RNA of the conjugate comprises RNA  
20 effective for stimulating signaling by TLR3, TLR7, TLR8, or any combination thereof.
61. The composition according to claim 60, wherein the conjugate comprises a chimeric  
DNA:RNA backbone.
- 25 62. The composition according to claim 61, wherein the chimeric backbone comprises a  
cleavage site between the DNA and the RNA.
63. The composition according to claim 60, wherein the conjugate comprises a double-  
stranded DNA:RNA heteroduplex.
- 30 64. The composition according to claim 60, further comprising a pharmaceutically  
acceptable carrier.

65. A method for stimulating TLR8 signaling, comprising:  
contacting TLR8 with an isolated RNA in an effective amount to stimulate TLR8  
signaling.
- 5
66. The method according to claim 65, wherein the RNA is double-stranded RNA.
67. The method according to claim 65, wherein the RNA is ribosomal RNA.
- 10 68. The method according to claim 65, wherein the RNA is transfer RNA.
69. The method according to claim 65, wherein the RNA is messenger RNA.
70. The method according to claim 65, wherein the RNA is viral RNA.
- 15
71. The method according to claim 65, wherein the RNA is G,U-rich RNA.
72. The method according to claim 65, wherein the RNA consists essentially of G and U.
- 20 73. A method for stimulating TLR8 signaling, comprising:  
contacting TLR8 with a mixture of nucleosides consisting essentially of G and U in a  
ratio between 1G:50U and 10G:1U, in an amount effective to stimulate TLR8 signaling.
74. The method according to claim 73, wherein the nucleosides are ribonucleosides.
- 25
75. The method according to claim 73, wherein the nucleosides comprise a mixture of  
ribonucleosides and deoxyribonucleosides.
76. The method according to claim 73, wherein the G is a guanosine derivative selected  
30 from the group consisting of: 8-bromoguanosine, 8-oxoguanosine, 8-mercaptoguanosine,  
7-allyl-8-oxoguanosine, guanosine ribonucleoside vanadyl complex, inosine, and  
nebularine.

- 124 -

77. A method for stimulating TLR8 signaling, comprising:  
contacting TLR8 with a mixture of ribonucleoside vanadyl complexes.

5 78. The method according to claim 77, wherein the mixture comprises guanosine  
ribonucleoside vanadyl complexes.

79. A method for stimulating TLR8 signaling, comprising:  
contacting TLR8 with an isolated G,U-rich oligonucleotide comprising a sequence  
10 selected from the group consisting of: UUGUGG, UGGUUG, GUGUGU, and  
GGGUUU, in an amount effective to stimulate TLR8 signaling.

80. The method according to claim 79, wherein the oligonucleotide is an  
oligoribonucleotide.

15 81. The method according to claim 79, wherein the oligonucleotide is 7-50 bases long.

82. The method according to claim 79, wherein the oligonucleotide is 12-24 bases long.

20 83. The method according to claim 79, wherein the oligonucleotide has a sequence  
5'-GUUGUGGUUGUGGUUGUG-3' (SEQ ID NO:1).

84. A method for stimulating TLR8 signaling, comprising:  
contacting TLR8 with an at least partially double-stranded nucleic acid molecule  
25 comprising at least one G-U base pair, in an amount effective to stimulate TLR8  
signaling.

85. A method for supplementing a TLR8-mediated immune response, comprising:  
contacting TLR8 with an effective amount of a TLR8 ligand to induce a TLR8-  
30 mediated immune response; and  
contacting a TLR other than TLR8 with an effective amount of a ligand for the TLR  
other than TLR8 to induce an immune response mediated by the TLR other than TLR8.

- 125 -

86. A method for supplementing a TLR8-mediated immune response in a subject, comprising:  
administering to a subject in need of an immune response an effective amount of a  
5 TLR8 ligand to induce a TLR8-mediated immune response; and  
administering to the subject an effective amount of a ligand for a TLR other than TLR8 to induce an immune response mediated by the TLR other than TLR8.
87. The method according to claim 86, wherein the TLR other than TLR8 is TLR9.  
10
88. The method according to claim 87, wherein the ligand for TLR9 is a CpG nucleic acid.
89. The method according to claim 88, wherein the CpG nucleic acid has a stabilized  
15 backbone.
90. The method according to claim 87, wherein the ligand for TLR8 and the ligand for TLR9 are a conjugate.
- 20 91. The method according to claim 90, wherein the conjugate comprises a double-stranded DNA:RNA heteroduplex.
92. The method according to claim 90, wherein the conjugate comprises a chimeric DNA:RNA backbone.  
25
93. The method according to claim 92, wherein the chimeric backbone comprises a cleavage site between the DNA and the RNA.
94. A method for stimulating TLR7 signaling, comprising:  
30 contacting TLR7 with an isolated guanosine ribonucleoside in an effective amount to stimulate TLR7 signaling.

- 126 -

95. The method according to claim 94, wherein the guanosine ribonucleoside is a guanosine ribonucleoside derivative selected from the group consisting of: 8-bromoguanosine, 8-oxoguanosine, 8-mercaptoguanosine, 7-allyl-8-oxoguanosine, guanosine ribonucleoside vanadyl complex, inosine, and nebularine.
- 5 96. The method according to claim 95, wherein the guanosine ribonucleoside derivative is 8-oxoguanosine.
97. The method according to claim 94, wherein the guanosine nucleoside is a  
10 ribonucleoside.
98. The method according to claim 97, wherein the guanosine nucleoside comprises a mixture of ribonucleosides and deoxyribonucleosides.
- 15 99. A method for stimulating TLR7 signaling, comprising:  
contacting TLR7 with an isolated nucleic acid comprising a terminal oxidized or halogenized guanosine in an effective amount to stimulate TLR7 signaling.
100. The method according to claim 99, wherein the oxidized or halogenized guanosine is  
20 8-oxoguanosine
101. A method for stimulating TLR7 signaling, comprising:  
contacting TLR7 with an isolated RNA in an effective amount to stimulate TLR7  
signaling.
- 25 102. The method according to claim 101, wherein the RNA is double-stranded RNA.
103. The method according to claim 101, wherein the RNA is ribosomal RNA.
- 30 104. The method according to claim 101, wherein the RNA is transfer RNA.
105. The method according to claim 101, wherein the RNA is messenger RNA.

- 127 -

106. The method according to claim 101, wherein the RNA is viral RNA.
107. The method according to claim 101, wherein the RNA is G-rich RNA.
- 5 108. The method according to claim 107, further wherein the RNA is part of a DNA:RNA heteroduplex.
109. The method according to claim 107, wherein the RNA consists essentially of  
10 guanosine ribonucleoside.
110. A method for stimulating TLR7 signaling, comprising:  
contacting TLR7 with a mixture of ribonucleoside vanadyl complexes.
- 15 111. The method according to claim 110, wherein the mixture comprises guanosine ribonucleoside vanadyl complexes.
112. A method for supplementing a TLR7-mediated immune response, comprising:  
contacting TLR7 with an effective amount of a TLR7 ligand to induce a TLR7-  
20 mediated immune response; and  
contacting a TLR other than TLR7 with an effective amount of a ligand for the TLR other than TLR7 to induce an immune response mediated by the TLR other than TLR7.
113. A method for supplementing a TLR7-mediated immune response in a subject,  
25 comprising:  
administering to a subject in need of an immune response an effective amount of a TLR7 ligand to induce a TLR7-mediated immune response; and  
administering to the subject an effective amount of a ligand for a TLR other than TLR7 to induce an immune response mediated by the TLR other than TLR7.
- 30 114. The method according to claim 113, wherein the TLR other than TLR7 is TLR9.

- 128 -

115. The method according to claim 114, wherein the ligand for TLR9 is a CpG nucleic acid.

116. The method according to claim 115, wherein the CpG nucleic acid has a stabilized  
5 backbone.

117. The method according to claim 114, wherein the ligand for TLR7 and the ligand for TLR9 are a conjugate.

10 118. The method according to claim 117, wherein the conjugate comprises a double-stranded DNA:RNA heteroduplex.

119. The method according to claim 117, wherein the conjugate comprises a chimeric DNA:RNA backbone.

15 120. The method according to claim 119, wherein the chimeric backbone comprises a cleavage site between the DNA and the RNA.

121. A method for screening candidate immunostimulatory compounds, comprising:  
20 measuring a TLR8-mediated reference signal in response to an RNA reference;  
measuring a TLR8-mediated test signal in response to a candidate immunostimulatory compound; and  
comparing the TLR8-mediated test signal to the TLR8-mediated reference signal.

25 122. A method for screening candidate immunostimulatory compounds, comprising:  
measuring a TLR8-mediated reference signal in response to an imidazoquinoline reference;  
measuring a TLR8-mediated test signal in response to a candidate immunostimulatory compound; and  
30 comparing the TLR8-mediated test signal to the TLR8-mediated reference signal.

- 129 -

123. The method according to claim 122, wherein the imidazoquinoline is resiquimod (R-848).
124. The method according to claim 122, wherein the imidazoquinoline is imiquimod  
5 (R-837).
125. A method for screening candidate immunostimulatory compounds, comprising:  
measuring a TLR7-mediated reference signal in response to a 7-allyl-8-oxoguanosine  
reference;  
10 measuring a TLR7-mediated test signal in response to a candidate immunostimulatory  
compound; and  
comparing the TLR7-mediated test signal to the TLR7-mediated reference signal.
126. A method for screening candidate immunostimulatory compounds, comprising:  
15 measuring a TLR7-mediated reference signal in response to an imidazoquinoline  
reference;  
measuring a TLR7-mediated test signal in response to a candidate immunostimulatory  
compound; and  
comparing the TLR7-mediated test signal to the TLR7-mediated reference signal.  
20
127. The method according to claim 126, wherein the imidazoquinoline is resiquimod (R-848).
128. The method according to claim 126, wherein the imidazoquinoline is imiquimod  
25 (R-837).

1/19

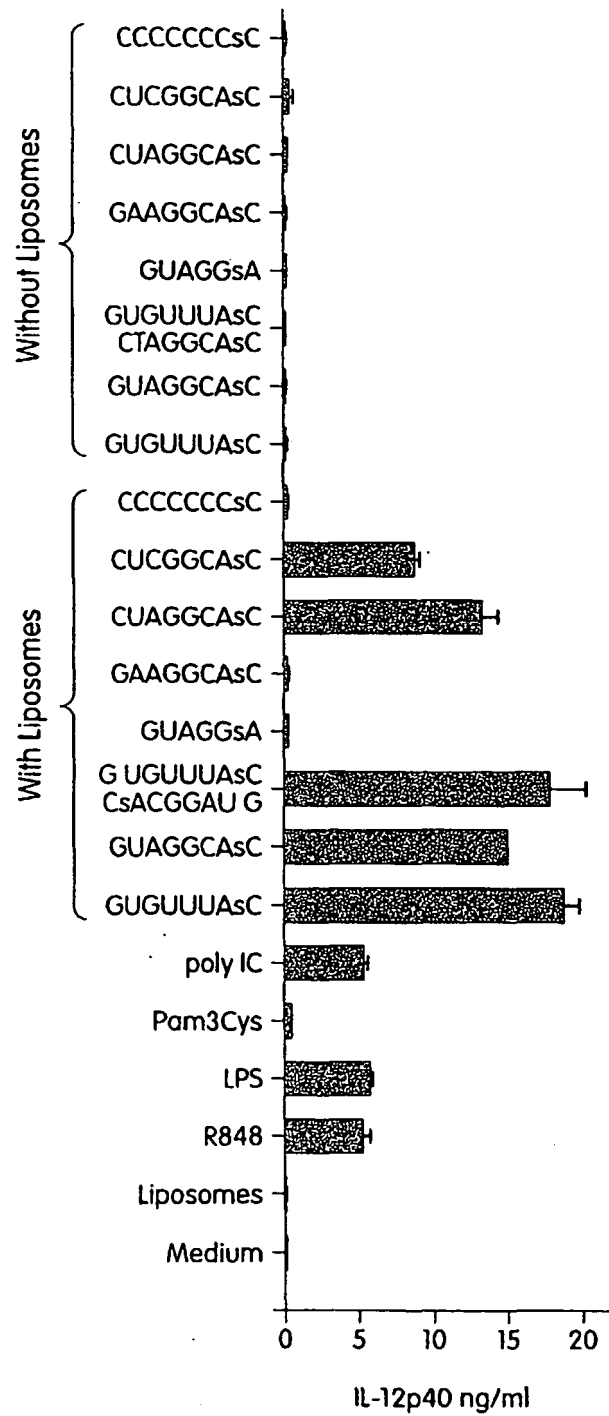


Fig. 1

2/19

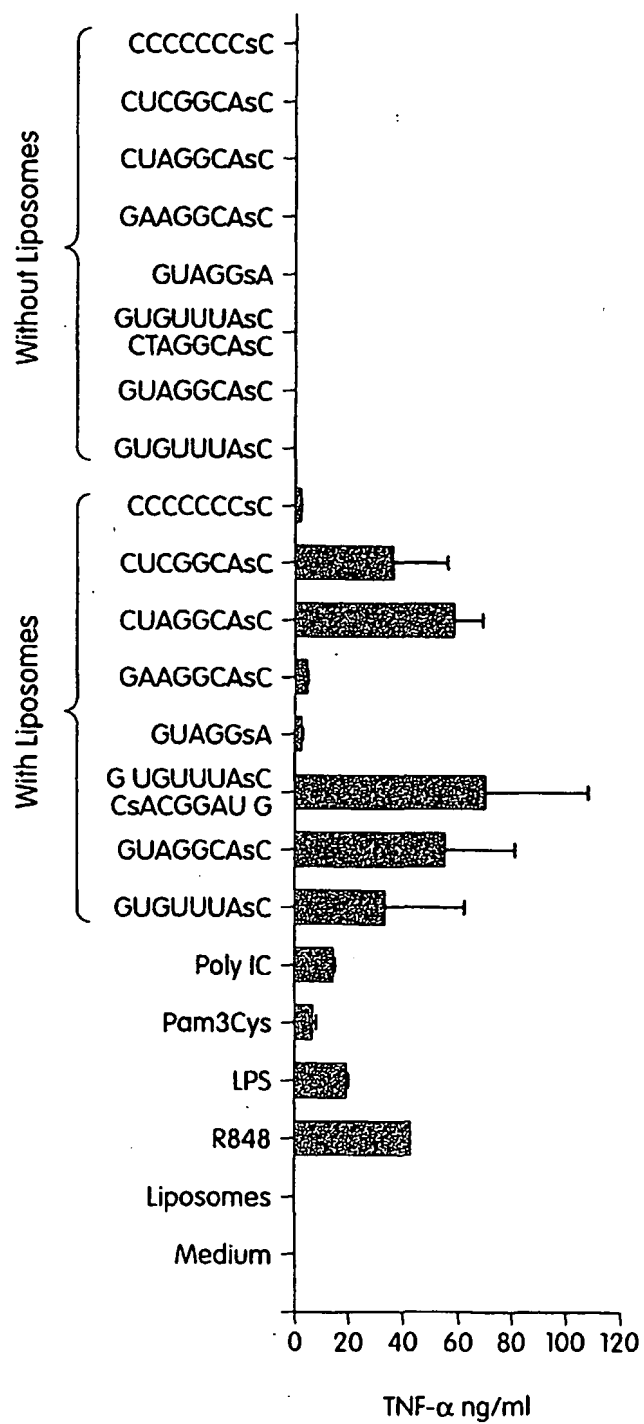


Fig. 2

3/19

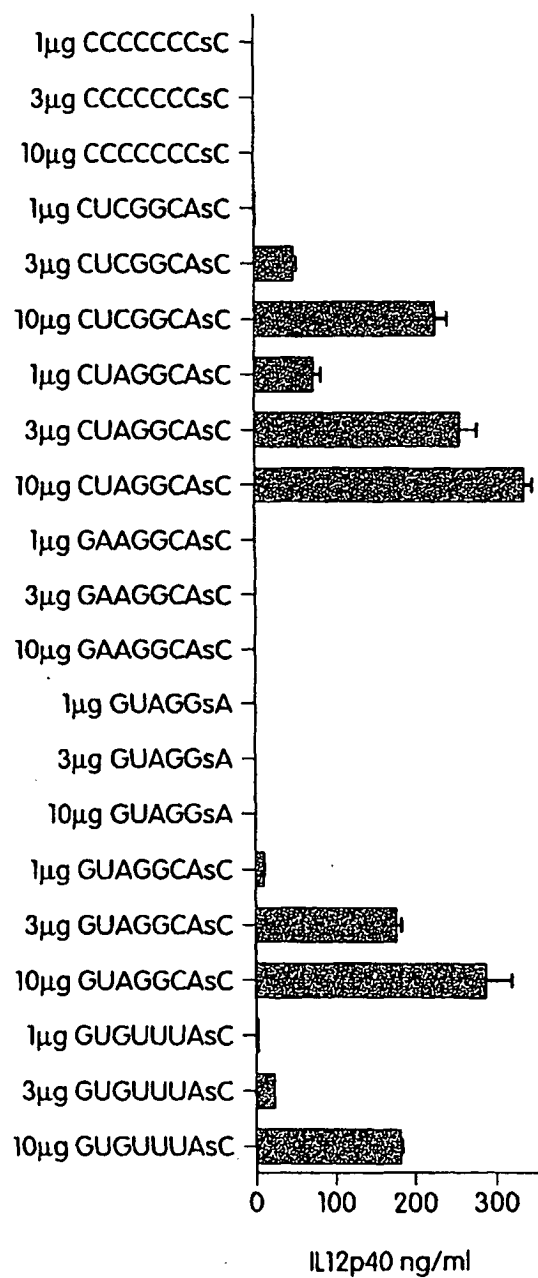


Fig. 3

4/19

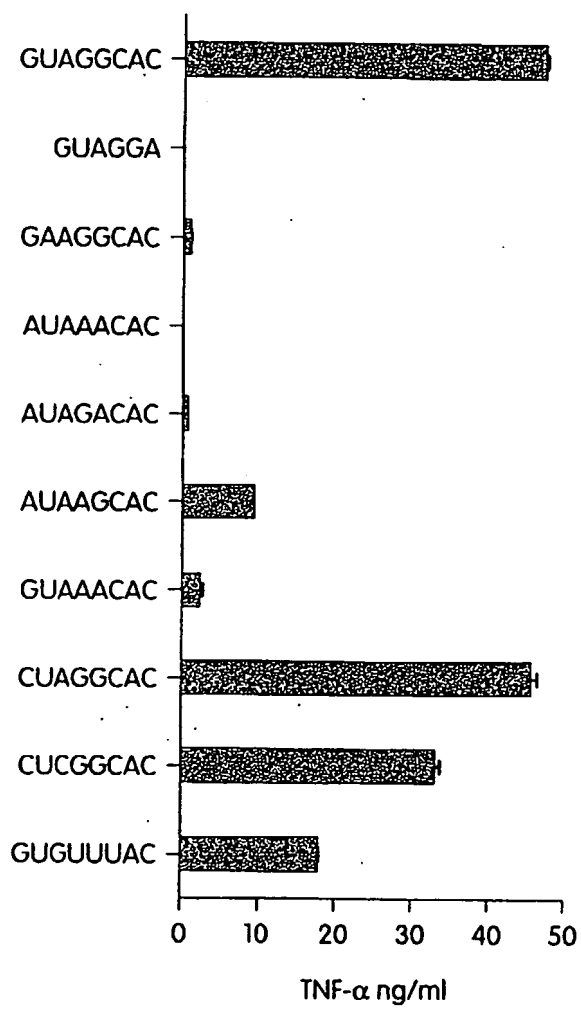


Fig. 4

5/19

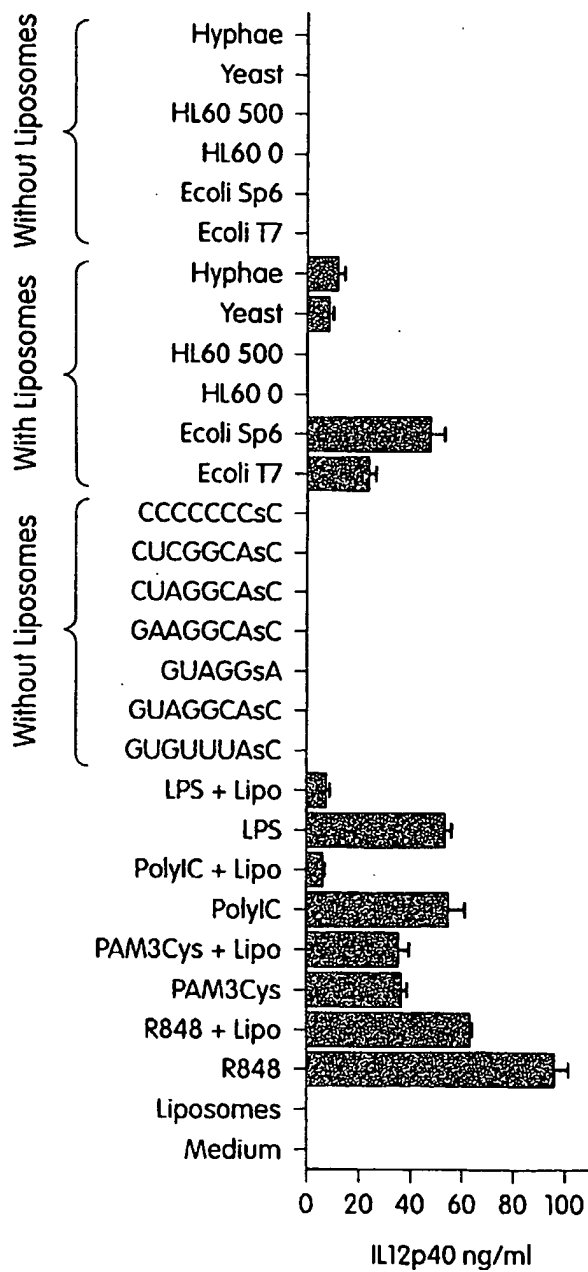


Fig. 5

6/19

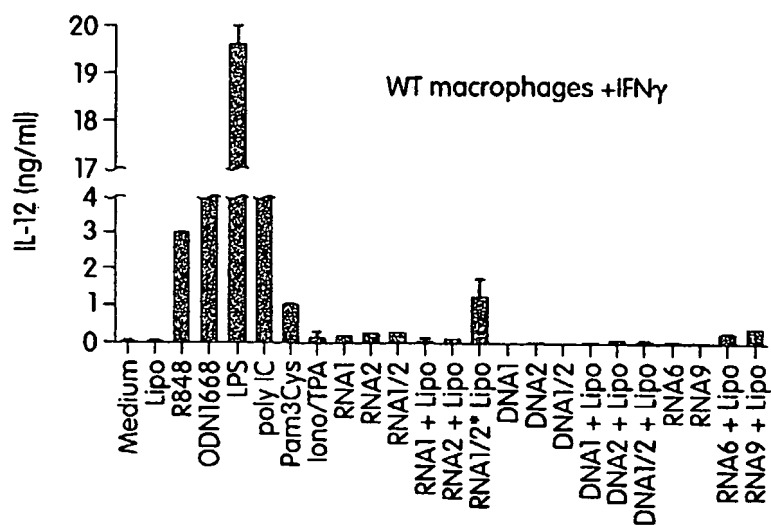


Fig. 6A

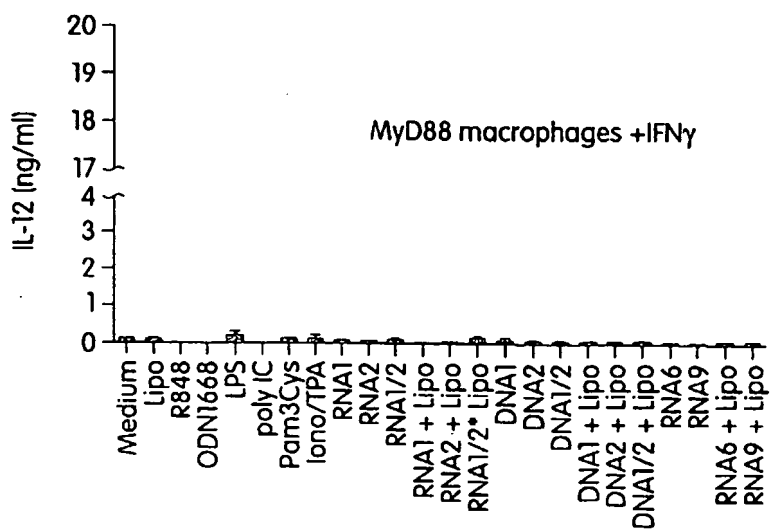


Fig. 6B

7/19

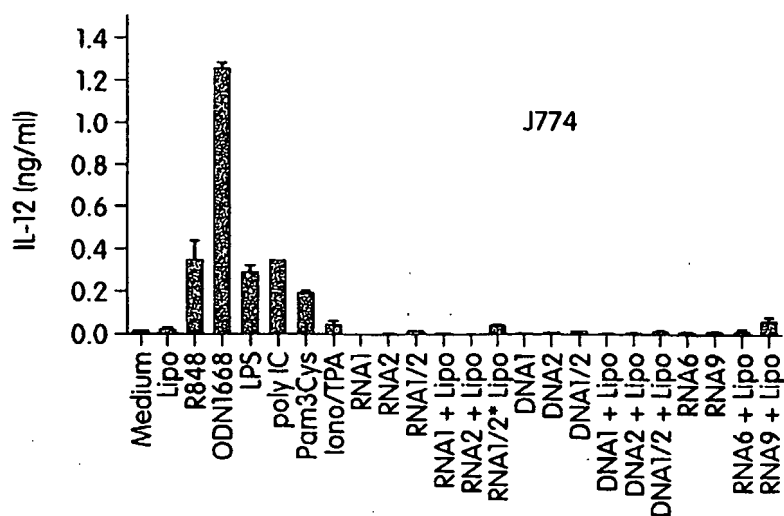


Fig. 6C

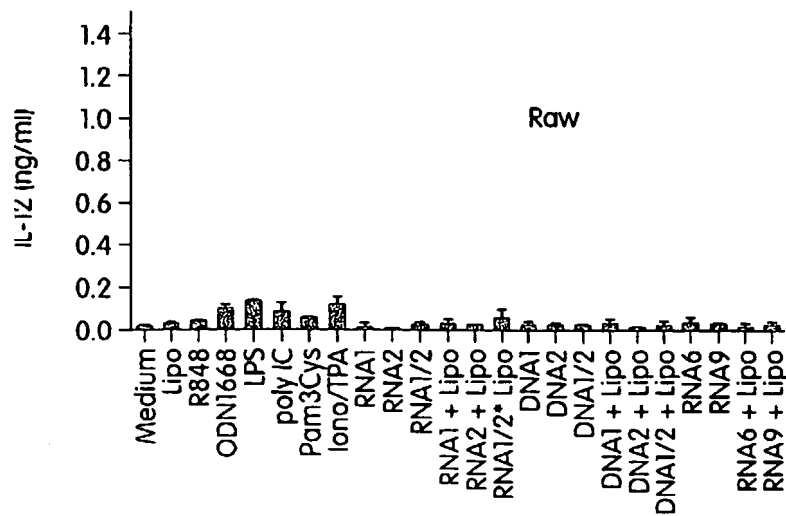


Fig. 6D

8/19

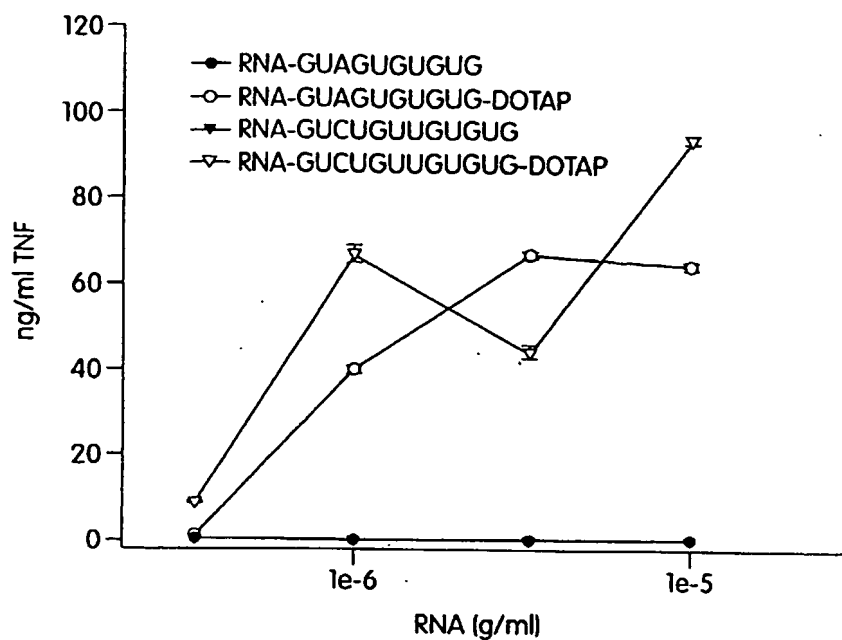


Fig. 7A

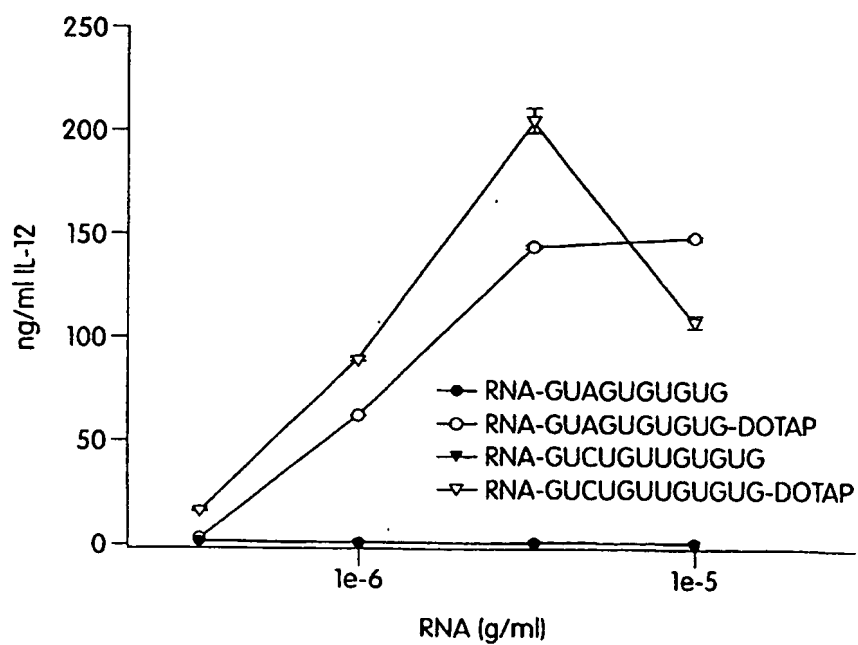


Fig. 7B

9/19

HOMOLOGY AMONG TOLL-LIKE RECEPTORS

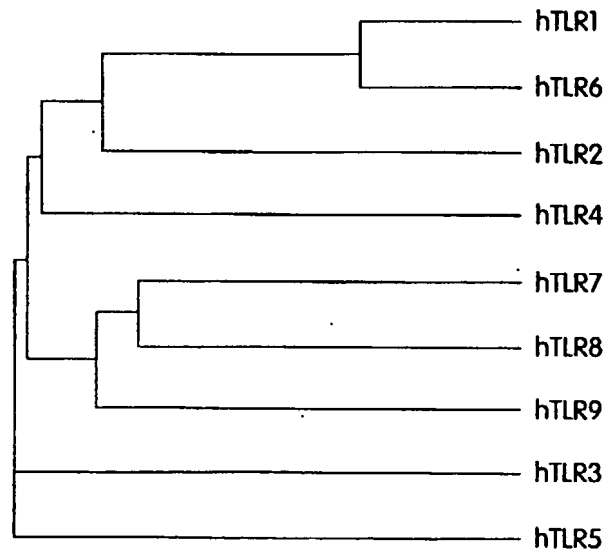


Fig. 8

10/19

TLR7 AND TLR8 HAVE TLR9-LIKE  
NUCLEIC ACID BINDING DOMAINS

CXXC motif	GNCXXCXXXXXXXXCXXC
Human TLR9	GNCRRCDHAPNPCMEC
Murine TLR9	GNCRRCDHAPNPCMIC
Human TLR8	GNCPRCFNAPFPCVPC
Human TLR7	GNCPRCYNAPFPCAPC

MBD motif		*	*	*	*	*		*	*
MBD-1	V-X-R-XXXX-T-XX-R-X- <u>D-X-Y</u> -XXXXXXXXXX-R-S								
hTLR9	G-X-Q-XXXX-S-XX-K-X- <u>D-X-Y</u> -XXXXXXXXXX-R-L								
hTLR8	H-X-K-XXXX-T-XX-R-X- <u>D-X-D</u> -XXXXXXXXXX-D-L								
hTLR7	E-X-R-XXXX-S-XX-R-X- <u>D-X-L</u> -XXXXXXXXXX-K-L								

Fig. 9

11/19

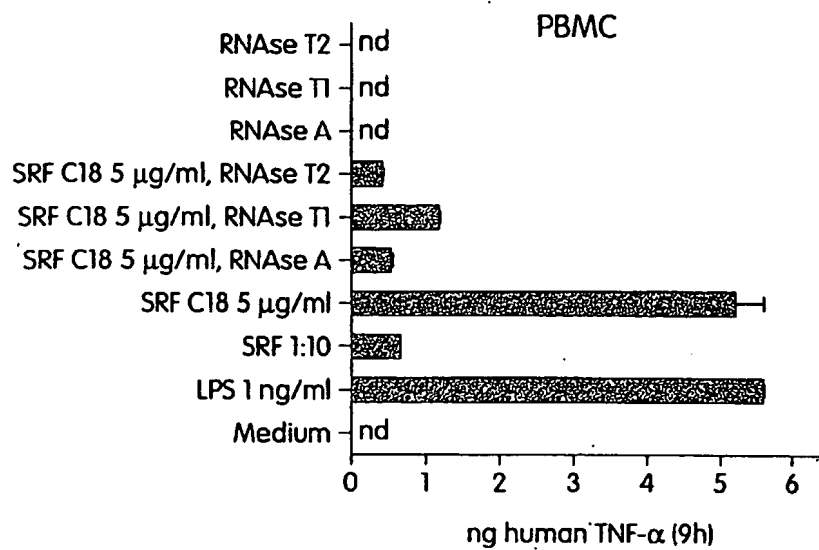


Fig. 10

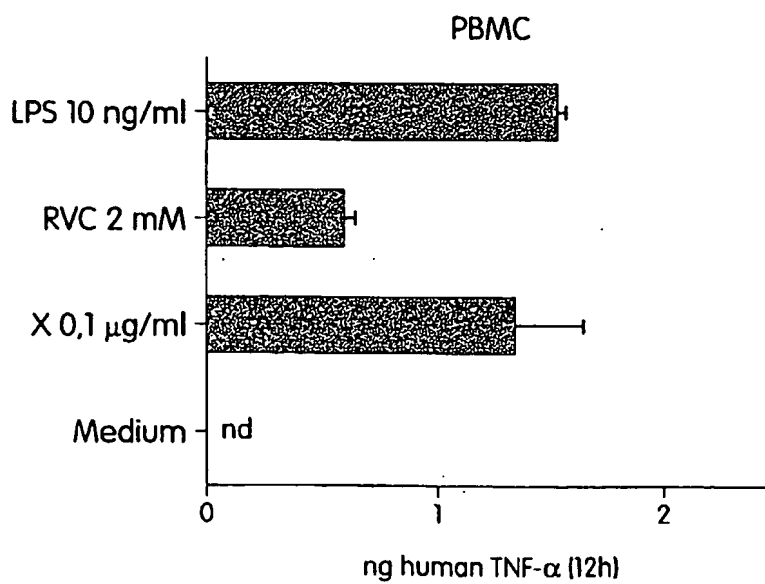


Fig. 11

12/19

Fig. 12

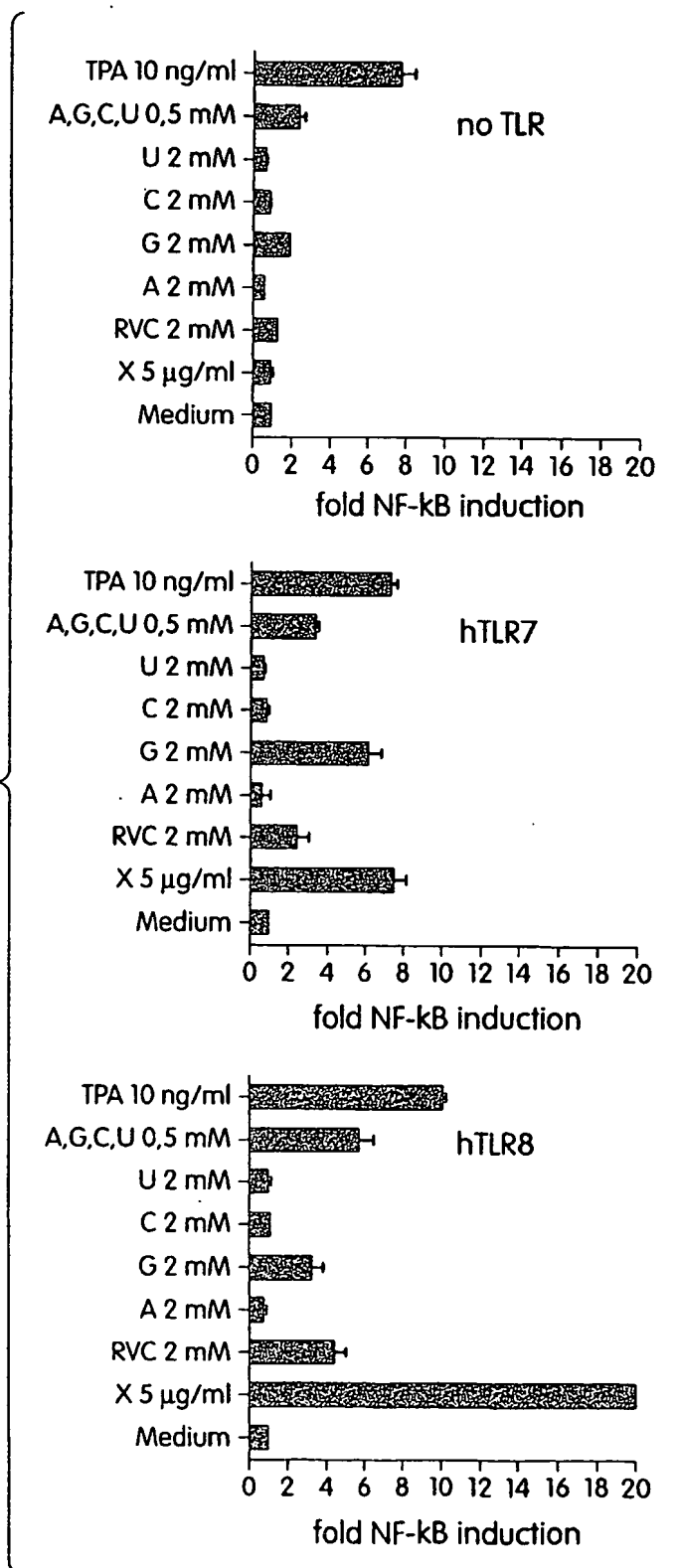
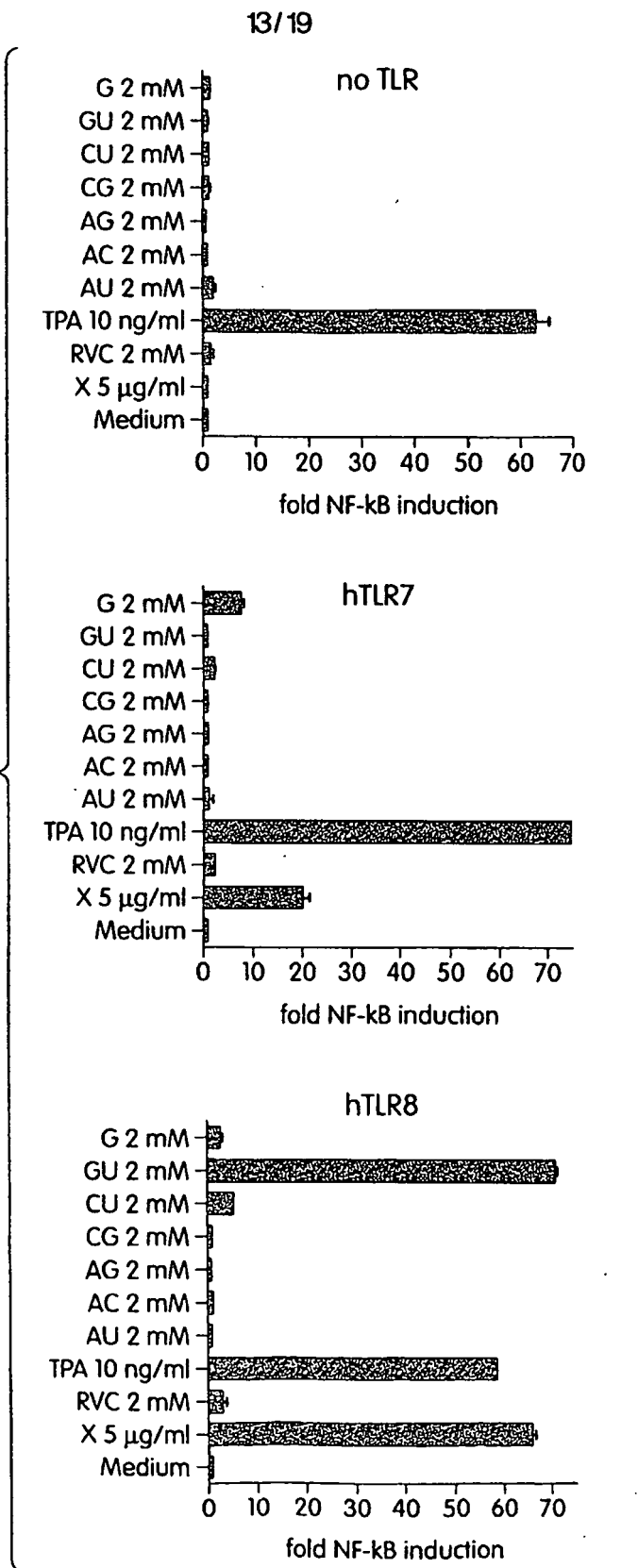


Fig. 13



14/19

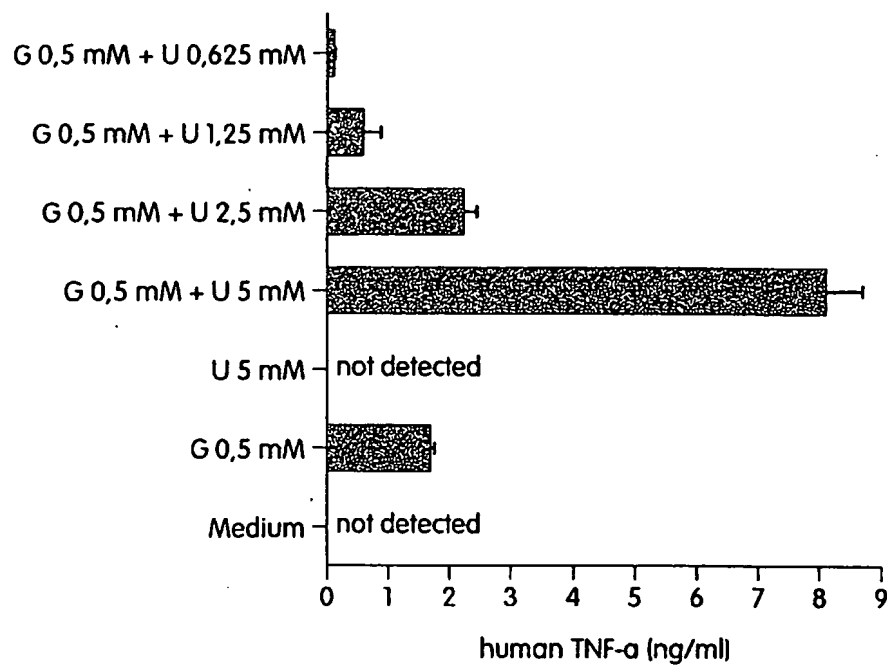


Fig. 14

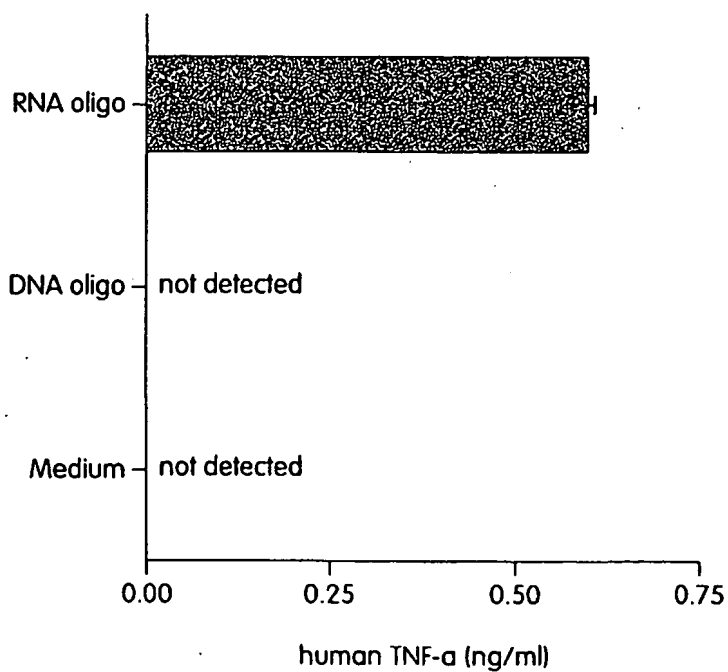


Fig. 15

15/19

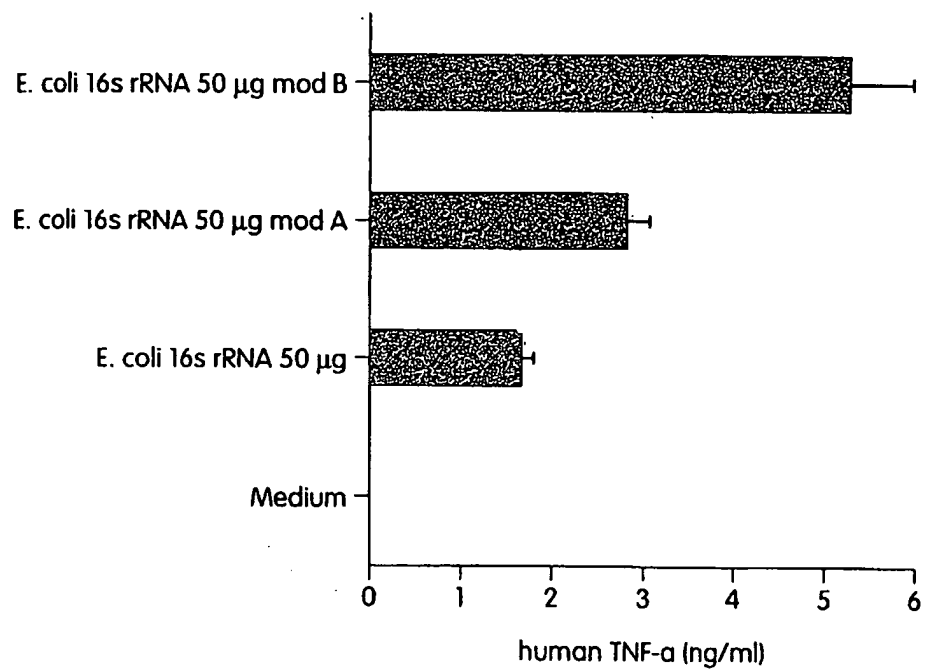
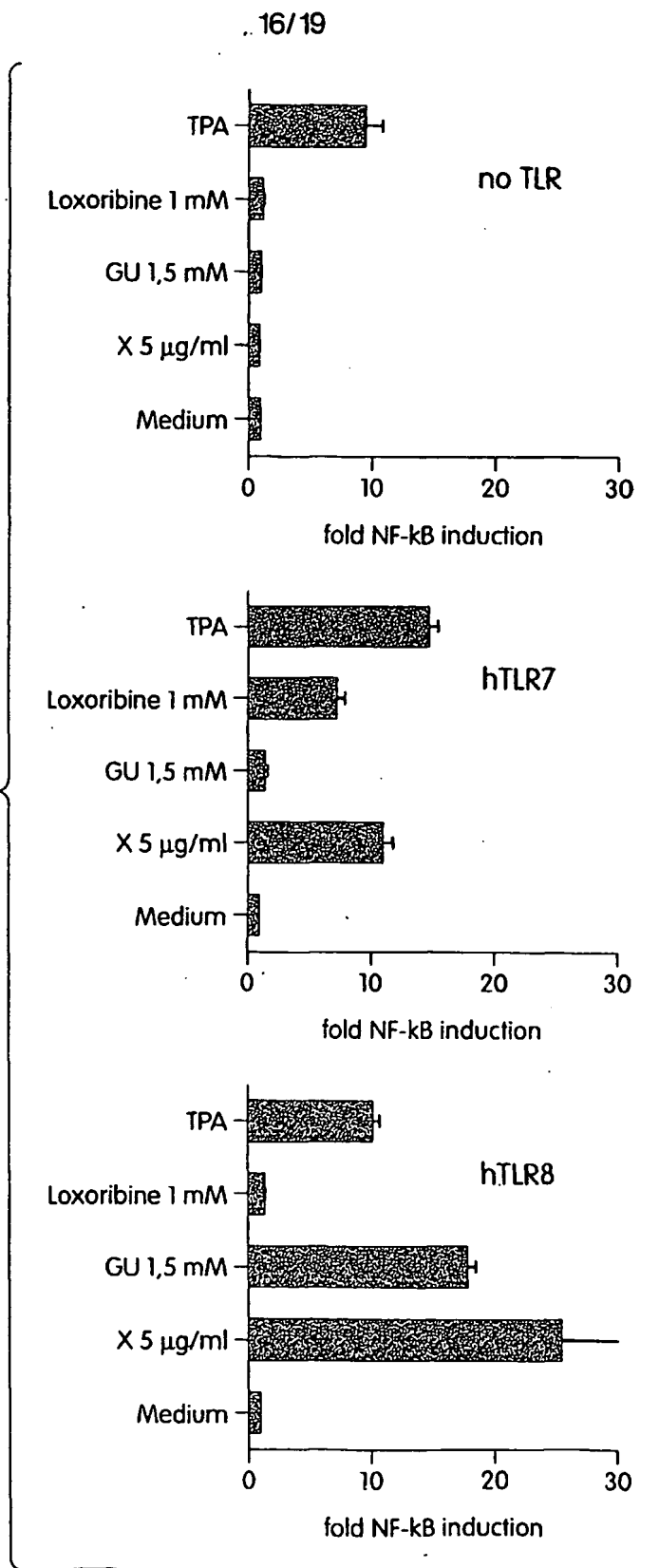


Fig. 16

Fig. 17



17/19

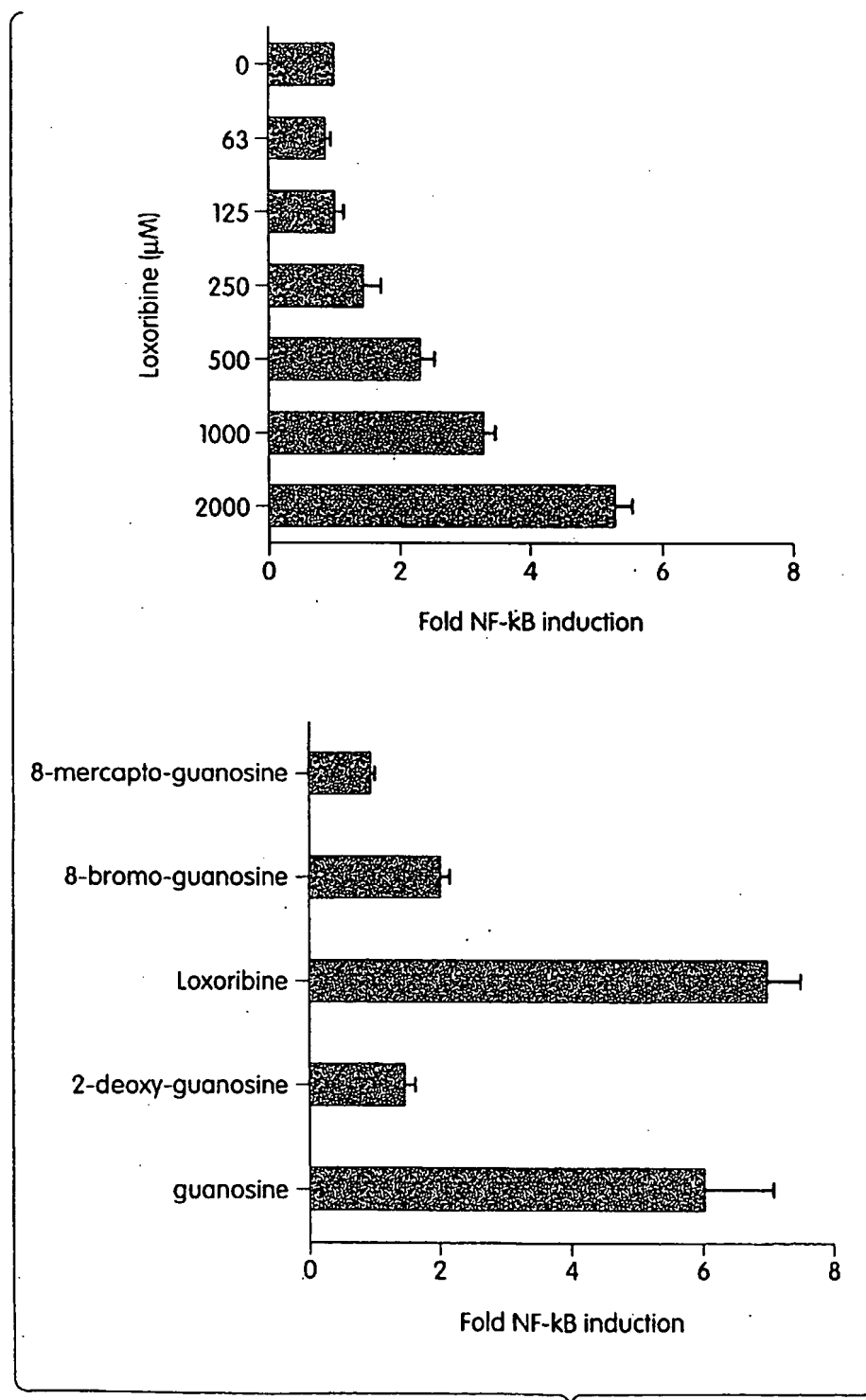


Fig. 18

18/19

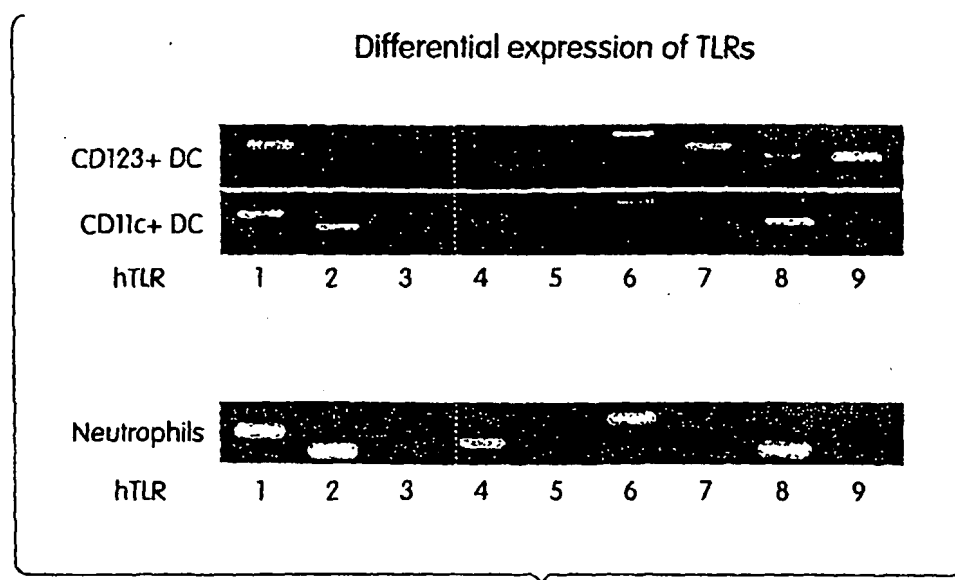
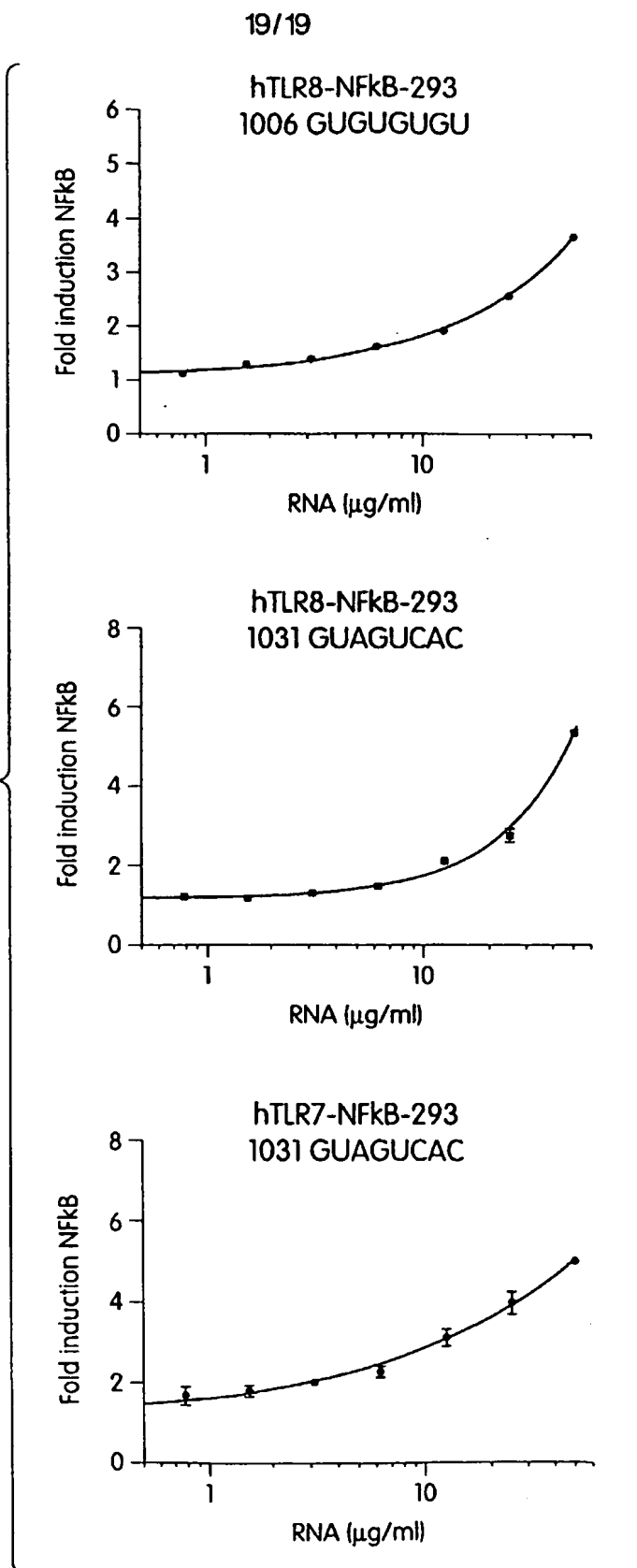


Fig. 19

Fig. 20



## SEQUENCE LISTING

<110> Coley Pharmaceutical GmbH

<120> Immunostimulatory G,U-Containing Oligoribonucleotides

<130> C01041.70037

<150> US 60/421,966

<151> 2002-10-29

<150> US 60/370,515

<151> 2002-04-04

<160> 39

<170> PatentIn version 3.1

<210> 1

<211> 18

<212> RNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 1  
guugugguug ugguugug 18

<210> 2

<211> 10

<212> RNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 2  
guagugugug 10

<210> 3

<211> 12

<212> RNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 3  
gucuguugug ug 12

<210> 4

<211> 27

<212> RNA

<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<400> 4  
gccgaguagu guugggucgc gaaaggc 27

<210> 5  
<211> 33  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<400> 5  
attgaagagt ttgatcatgg ctcagattga acg 33

<210> 6  
<211> 27  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<400> 6  
taaggaggtg atccaaccgc aggttcc 27

<210> 7  
<211> 20  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<400> 7  
tccatgacgt tcctgatgct 20

<210> 8  
<211> 26  
<212> RNA  
<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<400> 8  
uccgcaaugg acgaaagucu gacgga 26

<210> 9  
<211> 26  
<212> RNA  
<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<400> 9  
gagaugggug cgagagcguc aguauu 26

<210> 10  
<211> 40  
<212> RNA  
<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<400> 10  
cacacacugc uuaagcgcuu gccugcuuaa guagugugug 40

<210> 11  
<211> 14  
<212> RNA  
<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<400> 11  
gugugugugg gggg 14

<210> 12  
<211> 13  
<212> RNA  
<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<400> 12  
ggggggugug ugu 13

<210> 13  
<211> 14  
<212> RNA  
<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<220>  
<221> misc\_feature  
<222> (9)..(14)  
<223> deoxyT

<400> 13

gugugugunn nnnn

14

<210> 14  
<211> 13  
<212> RNA  
<213> Artificial sequence  
  
<220>  
<223> Synthetic oligonucleotide  
  
<220>  
<221> misc\_feature  
<222> (1)..(5)  
<223> deoxyT

<400> 14  
nnnnngugug ugu

13

<210> 15  
<211> 24  
<212> DNA  
<213> Artificial sequence  
  
<220>  
<223> Synthetic oligonucleotide

<400> 15  
cacctctcat gctctgctct cttc

24

<210> 16  
<211> 25  
<212> DNA  
<213> Artificial sequence  
  
<220>  
<223> Synthetic oligonucleotide

<400> 16  
gctagaccgt ttccttgaac acctg

25

<210> 17  
<211> 25  
<212> DNA  
<213> Artificial sequence  
  
<220>  
<223> Synthetic oligonucleotide

<400> 17  
ctgcgctgct gcaagttacg gaatg

25

<210> 18  
<211> 25

<212> DNA  
<213> Artificial sequence

<220>  
<223> Synthetic oligonucleotide

<400> 18  
gcgcgaaatc atgacttaac gtcag

25

<210> 19  
<211> 18  
<212> DNA  
<213> Artificial sequence  
  
<220>  
<223> Synthetic oligonucleotide

<220>  
<221> misc\_feature  
<222> (2)..(3)  
<223> U

<220>  
<221> misc\_feature  
<222> (5)..(5)  
<223> U

<220>  
<221> misc\_feature  
<222> (8)..(9)  
<223> U

<220>  
<221> misc\_feature  
<222> (11)..(11)  
<223> U

<220>  
<221> misc\_feature  
<222> (14)..(15)  
<223> U

<220>  
<221> misc\_feature  
<222> (17)..(17)  
<223> U

<400> 19  
gnngnggng nggngngng

18

<210> 20

&lt;211&gt; 904

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 20

Met Arg Gln Thr Leu Pro Cys Ile Tyr Phe Trp Gly Gly Leu Leu Pro  
 1 5 10 15

Phe Gly Met Leu Cys Ala Ser Ser Thr Thr Lys Cys Thr Val Ser His  
 20 25 30

Glu Val Ala Asp Cys Ser His Leu Lys Leu Thr Gln Val Pro Asp Asp  
 35 40 45

Leu Pro Thr Asn Ile Thr Val Leu Asn Leu Thr His Asn Gln Leu Arg  
 50 55 60

Arg Leu Pro Ala Ala Asn Phe Thr Arg Tyr Ser Gln Leu Thr Ser Leu  
 65 70 75 80

Asp Val Gly Phe Asn Thr Ile Ser Lys Leu Glu Pro Glu Leu Cys Gln  
 85 90 95

Lys Leu Pro Met Leu Lys Val Leu Asn Leu Gln His Asn Glu Leu Ser  
 100 105 110

Gln Leu Ser Asp Lys Thr Phe Ala Phe Cys Thr Asn Leu Thr Glu Leu  
 115 120 125

His Leu Met Ser Asn Ser Ile Gln Lys Ile Lys Asn Asn Pro Phe Val  
 130 135 140

Lys Gln Lys Asn Leu Ile Thr Leu Asp Leu Ser His Asn Gly Leu Ser  
 145 150 155 160

Ser Thr Lys Leu Gly Thr Gln Val Gln Leu Glu Asn Leu Gln Glu Leu  
 165 170 175

Leu Leu Ser Asn Asn Lys Ile Gln Ala Leu Lys Ser Glu Glu Leu Asp  
 180 185 190

Ile Phe Ala Asn Ser Ser Leu Lys Lys Leu Glu Leu Ser Ser Asn Gln  
 195 200 205

Ile Lys Glu Phe Ser Pro Gly Cys Phe His Ala Ile Gly Arg Leu Phe

210	215	220
Gly Leu Phe Leu Asn Asn Val Gln Leu Gly Pro Ser Leu Thr Glu Lys		
225	230	235 240
Leu Cys Leu Glu Leu Ala Asn Thr Ser Ile Arg Asn Leu Ser Leu Ser		
	245	250 255
Asn Ser Gln Leu Ser Thr Thr Ser Asn Thr Thr Phe Leu Gly Leu Lys		
	260	265 270
Trp Thr Asn Leu Thr Met Leu Asp Leu Ser Tyr Asn Asn Leu Asn Val		
	275	280 285
Val Gly Asn Asp Ser Phe Ala Trp Leu Pro Gln Leu Glu Tyr Phe Phe		
	290	295 300
Leu Glu Tyr Asn Asn Ile Gln His Leu Phe Ser His Ser Leu His Gly		
305	310	315 320
Leu Phe Asn Val Arg Tyr Leu Asn Leu Lys Arg Ser Phe Thr Lys Gln		
	325	330 335
Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile Asp Asp Phe Ser Phe Gln		
	340	345 350
Trp Leu Lys Cys Leu Glu His Leu Asn Met Glu Asp Asn Asp Ile Pro		
	355	360 365
Gly Ile Lys Ser Asn Met Phe Thr Gly Leu Ile Asn Leu Lys Tyr Leu		
	370	375 380
Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg Thr Leu Thr Asn Glu Thr		
385	390	395 400
Phe Val Ser Leu Ala His Ser Pro Leu His Ile Leu Asn Leu Thr Lys		
	405	410 415
Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala Phe Ser Trp Leu Gly His		
	420	425 430
Leu Glu Val Leu Asp Leu Gly Leu Asn Glu Ile Gly Gln Glu Leu Thr		
	435	440 445

Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile Phe Glu Ile Tyr Leu Ser  
 450 455 460

Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn Ser Phe Ala Leu Val Pro  
 465 470 475 480

Ser Leu Gln Arg Leu Met Leu Arg Arg Val Ala Leu Lys Asn Val Asp  
 485 490 495

Ser Ser Pro Ser Pro Phe Gln Pro Leu Arg Asn Leu Thr Ile Leu Asp  
 500 505 510

Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn Asp Asp Met Leu Glu Gly  
 515 520 525

Leu Glu Lys Leu Glu Ile Leu Asp Leu Gln His Asn Asn Leu Ala Arg  
 530 535 540

Leu Trp Lys His Ala Asn Pro Gly Gly Pro Ile Tyr Phe Leu Lys Gly  
 545 550 555 560

Leu Ser His Leu His Ile Leu Asn Leu Glu Ser Asn Gly Phe Asp Glu  
 565 570 575

Ile Pro Val Glu Val Phe Lys Asp Leu Phe Glu Leu Lys Ile Ile Asp  
 580 585 590

Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro Ala Ser Val Phe Asn Asn  
 595 600 605

Gln Val Ser Leu Lys Ser Leu Asn Leu Gln Lys Asn Leu Ile Thr Ser  
 610 615 620

Val Glu Lys Lys Val Phe Gly Pro Ala Phe Arg Asn Leu Thr Glu Leu  
 625 630 635 640

Asp Met Arg Phe Asn Pro Phe Asp Cys Thr Cys Glu Ser Ile Ala Trp  
 645 650 655

Phe Val Asn Trp Ile Asn Glu Thr His Thr Asn Ile Pro Glu Leu Ser  
 660 665 670

Ser His Tyr Leu Cys Asn Thr Pro Pro His Tyr His Gly Phe Pro Val  
 675 680 685

Arg Leu Phe Asp Thr Ser Ser Cys Lys Asp Ser Ala Pro Phe Glu Leu  
690 695 700

Phe Phe Met Ile Asn Thr Ser Ile Leu Leu Ile Phe Ile Phe Ile Val  
705 710 715 720

Leu Leu Ile His Phe Glu Gly Trp Arg Ile Ser Phe Tyr Trp Asn Val  
725 730 735

Ser Val His Arg Val Leu Gly Phe Lys Glu Ile Asp Arg Gln Thr Glu  
740 745 750

Gln Phe Glu Tyr Ala Ala Tyr Ile Ile His Ala Tyr Lys Asp Lys Asp  
755 760 765

Trp Val Trp Glu His Phe Ser Ser Met Glu Lys Glu Asp Gln Ser Leu  
770 775 780

Lys Phe Cys Leu Glu Glu Arg Asp Phe Glu Ala Gly Val Phe Glu Leu  
785 790 795 800

Glu Ala Ile Val Asn Ser Ile Lys Arg Ser Arg Lys Ile Ile Phe Val  
805 810 815

Ile Thr His His Leu Leu Lys Asp Pro Leu Cys Lys Arg Phe Lys Val  
820 825 830

His His Ala Val Gln Gln Ala Ile Glu Gln Asn Leu Asp Ser Ile Ile  
835 840 845

Leu Val Phe Leu Glu Glu Ile Pro Asp Tyr Lys Leu Asn His Ala Leu  
850 855 860

Cys Leu Arg Arg Gly Met Phe Lys Ser His Cys Ile Leu Asn Trp Pro  
865 870 875 880

Val Gln Lys Glu Arg Ile Gly Ala Phe Arg His Lys Leu Gln Val Ala  
885 890 895

Leu Gly Ser Lys Asn Ser Val His  
900

<210> 21  
<211> 3057

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 21

```

cacttttcgag agtgccgtct atttgccaca cacttccctg atgaaatgtc tggatttgga      60
ctaaagaaaa aaggaaaggc tagcagtcac ccaacagaat catgagacag actttgcctt      120
gtatctactt ttgggggggc cttttgcctt ttgggatgct gtgtgcatcc tccaccacca      180
agtgcactgt tagccatgaa gttgctgact gcagccacct gaagttgact caggtacccg      240
atgatctacc cacaacata acagtgttga accttaccac taatcaactc agaagattac      300
cagccgccaa cttcacaagg tatagccagc taactagctt ggatgtagga tttaacacca      360
tctcaaaact ggagccagaa ttgtgccaga aacttcccat gttaaaagtt ttgaacctcc      420
agcacaatga gctatctcaa ctttctgata aaacctttgc cttctgcacg aatttgactg      480
aactccatct catgtccaac tcaatccaga aaattaaaaa taatcccttt gtcaagcaga      540
agaatttaac cacattagat ctgtctcata atggcttgtc atctacaaaa ttaggaactc      600
aggttcagct ggaaaatctc caagagcttc tattatcaaa caataaaatt caagcgctaa      660
aaagtgaaga actggatata tttgccatt catctttaa aaaattagag ttgtcatcga      720
atcaaattaa agagttttct ccagggtgtt ttcacgcaat tggaagatta tttggcctct      780
ttctgaacaa tgtccagctg ggtcccagcc ttacagagaa gctatgtttg gaattagcaa      840
acacaagcat tcggaatctg tctctgagta acagccagct gtccaccacc agcaatacaa      900
ctttcttggg actaaagtgg acaaactctc ctatgctcga tctttcctac aacaacttaa      960
atgtggttgg taacgattcc tttgcttggc ttccacaact agaataattc ttcctagagt     1020
ataataatat acagcatttg ttttctcact ctttgcacgg gcttttcaat gtgaggtacc     1080
tgaatttgaa acggtctttt actaaacaaa gtatttcctt tgctcactc cccaagattg     1140
atgatttttc ttttcagtgg ctaaaatggt tggagcacct taacatggaa gataatgata     1200
ttccaggcat aaaaagcaat atgttcacag gattgataaa cctgaaatac ttaagtctat     1260
ccaactcctt tacaagtttg cgaactttga caaatgaaac atttgatatc cttgctcatt     1320
ctcccttaca catactcaac ctaaccaaga ataaaatctc aaaaatagag agtgatgctt     1380
tctcttggtt gggccaccta gaagtacttg acctgggcct taatgaaatt gggcaagaac     1440
tcacaggcca ggaatggaga ggtctagaaa atattttcga aatctatctt tcctacaaca     1500
agtacctgca gctgactagg aactcctttg ctttgggtccc aagccttcaa cgactgatgc     1560
tccgaagggg ggcccttaaa aatgtggata gctctccttc accattccag cctcttcgta     1620
acttgaccat tctggatcta agcaacaaca acatagccaa cataaatgat gacatgttgg     1680

```

```

agggtcttga gaaactagaa attctcgatt tgcagcataa caacttagca cggtcttgga 1740
aacacgcaaa ccctgggtggg cccatttatt tcctaaaggg tctgtctcac ctccacatcc 1800
ttaacttgga gtccaacggc tttagacgaga tcccagttga ggtcttcaag gatttatttg 1860
aactaaagat catcgattta ggattgaata atttaaacac acttccagca tctgtcttta 1920
ataatcaggt gtctctaaag tcattgaacc ttcagaagaa tctcataaca tccgttgaga 1980
agaaggtttt cgggccagct ttcaggaacc tgactgagtt agatatgcgc tttaatccct 2040
ttgattgcac gtgtgaaagt attgcctggg ttgttaattg gattaacgag acccatacca 2100
acatccctga gctgtcaagc cactaccttt gcaacactcc acctcactat catgggttcc 2160
cagtgagact ttttgataca tcattcttgca aagacagtgc cccctttgaa ctctttttca 2220
tgatcaatac cagtatcctg ttgattttta tctttattgt acttctcatc cactttgagg 2280
gctggaggat atctttttat tggaatgttt cagtacatcg agttcttggg ttcaaagaaa 2340
tagacagaca gacagaacag ttgaaatag cagcatatat aattcatgcc tataaagata 2400
aggattgggt ctgggaacat ttctcttcaa tggaaaagga agaccaatct ctcaaatttt 2460
gtctggaaga aagggaacttt gaggcgggtg tttttgaact agaagcaatt gttaacagca 2520
tcaaagaag cagaaaaatt atttttgtta taacacacca tctattaaaa gaccattat 2580
gcaaaagatt caaggtacat catgcagttc aacaagctat tgaacaaaat ctggattcca 2640
ttatattggg tttccttgag gagattccag attataaact gaaccatgca ctctgtttgc 2700
gaagaggaat gtttaaactc cactgcatct tgaactggcc agttcagaaa gaacggatag 2760
gtgcctttcg tcataaattg caagtagcac ttggatccaa aaactctgta cattaaattt 2820
atttaaatat tcaattagca aaggagaaac ttctcaatt taaaaagttc tatggcaaat 2880
ttaagttttc cataaagggtg ttataatttg ttattcata tttgtaaag attatattct 2940
atcacaatta catctcttct aggaaaatgt gtctccttat ttcaggccta tttttgacaa 3000
ttgacttaat ttaccctaaa ataaaacata taagcacgta aaaaaaaaaa aaaaaaa 3057

```

```

<210> 22
<211> 905
<212> PRT
<213> Mus musculus

```

```

<400> 22

```

```

Met Lys Gly Cys Ser Ser Tyr Leu Met Tyr Ser Phe Gly Gly Leu Leu
1           5           10           15

```

Ser Leu Trp Ile Leu Leu Val Ser Ser Thr Asn Gln Cys Thr Val Arg  
 20 25 30

Tyr Asn Val Ala Asp Cys Ser His Leu Lys Leu Thr His Ile Pro Asp  
 35 40 45

Asp Leu Pro Ser Asn Ile Thr Val Leu Asn Leu Thr His Asn Gln Leu  
 50 55 60

Arg Arg Leu Pro Pro Thr Asn Phe Thr Arg Tyr Ser Gln Leu Ala Ile  
 65 70 75 80

Leu Asp Ala Gly Phe Asn Ser Ile Ser Lys Leu Glu Pro Glu Leu Cys  
 85 90 95

Gln Ile Leu Pro Leu Leu Lys Val Leu Asn Leu Gln His Asn Glu Leu  
 100 105 110

Ser Gln Ile Ser Asp Gln Thr Phe Val Phe Cys Thr Asn Leu Thr Glu  
 115 120 125

Leu Asp Leu Met Ser Asn Ser Ile His Lys Ile Lys Ser Asn Pro Phe  
 130 135 140

Lys Asn Gln Lys Asn Leu Ile Lys Leu Asp Leu Ser His Asn Gly Leu  
 145 150 155 160

Ser Ser Thr Lys Leu Gly Thr Gly Val Gln Leu Glu Asn Leu Gln Glu  
 165 170 175

Leu Leu Leu Ala Lys Asn Lys Ile Leu Ala Leu Arg Ser Glu Glu Leu  
 180 185 190

Glu Phe Leu Gly Asn Ser Ser Leu Arg Lys Leu Asp Leu Ser Ser Asn  
 195 200 205

Pro Leu Lys Glu Phe Ser Pro Gly Cys Phe Gln Thr Ile Gly Lys Leu  
 210 215 220

Phe Ala Leu Leu Leu Asn Asn Ala Gln Leu Asn Pro His Leu Thr Glu  
 225 230 235 240

Lys Leu Cys Trp Glu Leu Ser Asn Thr Ser Ile Gln Asn Leu Ser Leu  
 245 250 255

Ala Asn Asn Gln Leu Leu Ala Thr Ser Glu Ser Thr Phe Ser Gly Leu  
                   260                  265                  270

Lys Trp Thr Asn Leu Thr Gln Leu Asp Leu Ser Tyr Asn Asn Leu His  
                   275                  280                  285

Asp Val Gly Asn Gly Ser Phe Ser Tyr Leu Pro Ser Leu Arg Tyr Leu  
                   290                  295                  300

Ser Leu Glu Tyr Asn Asn Ile Gln Arg Leu Ser Pro Arg Ser Phe Tyr  
 305                  310                  315                  320

Gly Leu Ser Asn Leu Arg Tyr Leu Ser Leu Lys Arg Ala Phe Thr Lys  
                   325                  330                  335

Gln Ser Val Ser Leu Ala Ser His Pro Asn Ile Asp Asp Phe Ser Phe  
                   340                  345                  350

Gln Trp Leu Lys Tyr Leu Glu Tyr Leu Asn Met Asp Asp Asn Asn Ile  
                   355                  360                  365

Pro Ser Thr Lys Ser Asn Thr Phe Thr Gly Leu Val Ser Leu Lys Tyr  
                   370                  375                  380

Leu Ser Leu Ser Lys Thr Phe Thr Ser Leu Gln Thr Leu Thr Asn Glu  
 385                  390                  395                  400

Thr Phe Val Ser Leu Ala His Ser Pro Leu Leu Thr Leu Asn Leu Thr  
                   405                  410                  415

Lys Asn His Ile Ser Lys Ile Ala Asn Gly Thr Phe Ser Trp Leu Gly  
                   420                  425                  430

Gln Leu Arg Ile Leu Asp Leu Gly Leu Asn Glu Ile Glu Gln Lys Leu  
                   435                  440                  445

Ser Gly Gln Glu Trp Arg Gly Leu Arg Asn Ile Phe Glu Ile Tyr Leu  
                   450                  455                  460

Ser Tyr Asn Lys Tyr Leu Gln Leu Ser Thr Ser Ser Phe Ala Leu Val  
 465                  470                  475                  480

Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val Ala Leu Lys Asn Val  
                   485                  490                  495

Asp Ile Ser Pro Ser Pro Phe Arg Pro Leu Arg Asn Leu Thr Ile Leu  
500 505 510

Asp Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn Glu Asp Leu Leu Glu  
515 520 525

Gly Leu Glu Asn Leu Glu Ile Leu Asp Phe Gln His Asn Asn Leu Ala  
530 535 540

Arg Leu Trp Lys Arg Ala Asn Pro Gly Gly Pro Val Asn Phe Leu Lys  
545 550 555 560

Gly Leu Ser His Leu His Ile Leu Asn Leu Glu Ser Asn Gly Leu Asp  
565 570 575

Glu Ile Pro Val Gly Val Phe Lys Asn Leu Phe Glu Leu Lys Ser Ile  
580 585 590

Asn Leu Gly Leu Asn Asn Leu Asn Lys Leu Glu Pro Phe Ile Phe Asp  
595 600 605

Asp Gln Thr Ser Leu Arg Ser Leu Asn Leu Gln Lys Asn Leu Ile Thr  
610 615 620

Ser Val Glu Lys Asp Val Phe Gly Pro Pro Phe Gln Asn Leu Asn Ser  
625 630 635 640

Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr Cys Glu Ser Ile Ser  
645 650 655

Trp Phe Val Asn Trp Ile Asn Gln Thr His Thr Asn Ile Phe Glu Leu  
660 665 670

Ser Thr His Tyr Leu Cys Asn Thr Pro His His Tyr Tyr Gly Phe Pro  
675 680 685

Leu Lys Leu Phe Asp Thr Ser Ser Cys Lys Asp Ser Ala Pro Phe Glu  
690 695 700

Leu Leu Phe Ile Ile Ser Thr Ser Met Leu Leu Val Phe Ile Leu Val  
705 710 715 720

Val Leu Leu Ile His Ile Glu Gly Trp Arg Ile Ser Phe Tyr Trp Asn  
725 730 735

Val Ser Val His Arg Ile Leu Gly Phe Lys Glu Ile Asp Thr Gln Ala  
740 745 750

Glu Gln Phe Glu Tyr Thr Ala Tyr Ile Ile His Ala His Lys Asp Arg  
755 760 765

Asp Trp Val Trp Glu His Phe Ser Pro Met Glu Glu Gln Asp Gln Ser  
770 775 780

Leu Lys Phe Cys Leu Glu Glu Arg Asp Phe Glu Ala Gly Val Leu Gly  
785 790 795 800

Leu Glu Ala Ile Val Asn Ser Ile Lys Arg Ser Arg Lys Ile Ile Phe  
805 810 815

Val Ile Thr His His Leu Leu Lys Asp Pro Leu Cys Arg Arg Phe Lys  
820 825 830

Val His His Ala Val Gln Gln Ala Ile Glu Gln Asn Leu Asp Ser Ile  
835 840 845

Ile Leu Ile Phe Leu Gln Asn Ile Pro Asp Tyr Lys Leu Asn His Ala  
850 855 860

Leu Cys Leu Arg Arg Gly Met Phe Lys Ser His Cys Ile Leu Asn Trp  
865 870 875 880

Pro Val Gln Lys Glu Arg Ile Asn Ala Phe His His Lys Leu Gln Val  
885 890 895

Ala Leu Gly Ser Arg Asn Ser Ala His  
900 905

<210> 23

<211> 3310

<212> DNA

<213> Mus musculus

<400> 23

tagaatatga tacagggatt gcaccataa tctgggctga atcatgaaag ggtgttcctc 60

ttatctaattg tactcctttg ggggactttt gtcctatgg attcttctgg tgtcttcac 120

aaaccaatgc actgtgagat acaacgtagc tgactgcagc catttgaagc taacacacat 180

acctgatgat cttccctcta acataacagt gttgaattt actcacaacc aactcagaag 240

attaccacct	accaacttta	caagatacag	ccaacttgct	atcttggatg	caggatttaa	300
ctccatttca	aaactggagc	cagaactgtg	ccaaatactc	cctttgttga	aagtattgaa	360
cctgcaacat	aatgagctct	ctcagatttc	tgatcaaacc	tttgtcttct	gcacgaacct	420
gacagaactc	gatctaattg	ctaactcaat	acacaaaatt	aaaagcaacc	ctttcaaaaa	480
ccagaagaat	ctaatacaat	tagatttgct	tcataatggt	ttatcatcta	caaagttggg	540
aacggggggtc	caactggaga	acctccaaga	actgctctta	gcaaaaaata	aaatccttgc	600
gttgcggaagt	gaagaacttg	agtttcttgg	caattcttct	ttacgaaagt	tggacttgtc	660
atcaaattcca	cttaaagagt	tctccccggg	gtgtttccag	acaattggca	agttattcgc	720
cctcctcttg	aacaacgccc	aactgaaccc	ccacctcaca	gagaagcttt	gctgggaact	780
ttcaaacaca	agcatccaga	atctctctct	ggctaacaac	cagctgctgg	ccaccagcga	840
gagcactttc	tctgggctga	agtggacaaa	tctcaccag	ctcgatcttt	cctacaacaa	900
cctccatgat	gtcggcaacg	gttccttctc	ctatctccca	agcctgaggt	atctgtctct	960
ggagtacaac	aatatacagc	gtctgtcccc	tcgctctttt	tatggactct	ccaacctgag	1020
gtacctgagt	ttgaagcgag	catttactaa	gcaaagtgtt	tcacttgctt	cacatcccaa	1080
cattgacgat	ttttcctttc	aatggttaaa	atatttggaa	tatctcaaca	tggatgacaa	1140
taatattcca	agtacccaaa	gcaatacctt	cacgggattg	gtgagtctga	agtacctaa	1200
tctttccaaa	actttcacaa	gtttgcaaac	tttaacaaat	gaaacatttg	tgtcacttgc	1260
tcattctccc	ttgctcactc	tcaacttaac	gaaaaatcac	atctcaaaaa	tagcaaatgg	1320
tactttctct	tggttaggcc	aactcaggat	acttgatctc	ggccttaatg	aaattgaaca	1380
aaaactcagc	ggccaggaat	ggagaggctc	gagaaatata	tttgagatct	acctatccta	1440
taacaaatac	ctccaactgt	ctaccagttc	ctttgcattg	gtccccagcc	ttcaaagact	1500
gatgctcagg	agggtgggcc	ttaaaaatgt	ggatatctcc	ccttcacctt	tccgcctctc	1560
tcgtaacttg	accattcttg	acttaagcaa	caacaacata	gccaacataa	atgaggactt	1620
gctggagggg	cttgagaatc	tagaaatcct	ggattttcag	cacaataact	tagccaggct	1680
ctggaaacgc	gcaaaccctg	gtgggtcccg	taatttcctg	aaggggctgt	ctcacctcca	1740
catcttgaat	ttagagtcca	acggcttaga	tgaaatccca	gtcgggggtt	tcaagaactt	1800
attcgaacta	aagagcatca	atctaggact	gaataactta	aacaaacttg	aaccattcat	1860
ttttgatgac	cagacatctc	taaggctcact	gaacctccag	aagaacctca	taacatctgt	1920
tgagaaggat	gttttcgggc	cgccttttca	aaacctgaac	agtttagata	tgcgcttcaa	1980
tccgttcgac	tgcacgtgtg	aaagtatttc	ctggtttggt	aactggatca	accagaccca	2040

```

cactaatatc tttgagctgt ccactcacta cctctgtaac actccacatc attattatgg 2100
cttccccctg aagcttttctg atacatcatc ctgtaaagac agcgccccct ttgaactcct 2160
cttcataatc agcaccagta tgctcctggt ttttatactt gtggtactgc tcattcacat 2220
cgagggctgg aggatctctt tttactggaa tgtttcagtg catcggattc ttggtttcaa 2280
ggaaatagac acacaggctg agcagtttga atatacagcc tacataattc atgcccataa 2340
agacagagac tgggtctggg aacattttct cccaatggaa gaacaagacc aatctctcaa 2400
atthttgccta gaagaaaggg actttgaagc aggcgtcctt ggacttgaag caattgttaa 2460
tagcatcaaa agaagccgaa aaatcatttt cgttatcaca caccatttat taaaagaccc 2520
tctgtgcaga agattcaagg tacatcacgc agttcagcaa gctattgagc aaaatctgga 2580
ttcaattata ctgatttttc tccagaatat tccagattat aaactaaacc atgcactctg 2640
tttgcaaga ggaatgttta aatctcattg catcttgaac tggccagttc agaaagaacg 2700
gataaatgcc tttcatcata aattgcaagt agcacttggg tctcggaatt cagcacatta 2760
aactcatttg aagatttggg gtcggtaaag ggatagatcc aatttataaa ggtccatcat 2820
gaatctaagt tttacttgaa agttttgtat atttatttat atgtatagat gatgatatta 2880
catcacaatc caatctcagt ttgaaatat ttcggcttat ttcattgaca tctggtttat 2940
tcactccaaa taaacacatg ggcagttaaa aacatcctct attaatagat taccatttaa 3000
ttcttgaggt gtatcacagc tttaaagggg tttaaatatt tttatataaa taagactgag 3060
agttttataa atgtaatttt ttaaaactcg agtcttactg tgtagctcag aaaggcctgg 3120
aaattaatat attagagagt catgtcttga acttatttat ctctgcctcc ctctgtctcc 3180
agagtgttgc ttttaagggc atgtagcacc acaccagct atgtacgtgt gggattttat 3240
aatgctcatt tttgagacgt ttatagaata aaagataatt gcttttatgg tataaggcta 3300
cttgaggtaa 3310

```

```

<210> 24
<211> 1049
<212> PRT
<213> Homo sapiens

```

```

<400> 24

```

```

Met Val Phe Pro Met Trp Thr Leu Lys Arg Gln Ile Leu Ile Leu Phe
1           5           10           15

```

```

Asn Ile Ile Leu Ile Ser Lys Leu Leu Gly Ala Arg Trp Phe Pro Lys
20           25           30

```

Thr Leu Pro Cys Asp Val Thr Leu Asp Val Pro Lys Asn His Val Ile  
 35 40 45

Val Asp Cys Thr Asp Lys His Leu Thr Glu Ile Pro Gly Gly Ile Pro  
 50 55 60

Thr Asn Thr Thr Asn Leu Thr Leu Thr Ile Asn His Ile Pro Asp Ile  
 65 70 75 80

Ser Pro Ala Ser Phe His Arg Leu Asp His Leu Val Glu Ile Asp Phe  
 85 90 95

Arg Cys Asn Cys Val Pro Ile Pro Leu Gly Ser Lys Asn Asn Met Cys  
 100 105 110

Ile Lys Arg Leu Gln Ile Lys Pro Arg Ser Phe Ser Gly Leu Thr Tyr  
 115 120 125

Leu Lys Ser Leu Tyr Leu Asp Gly Asn Gln Leu Leu Glu Ile Pro Gln  
 130 135 140

Gly Leu Pro Pro Ser Leu Gln Leu Leu Ser Leu Glu Ala Asn Asn Ile  
 145 150 155 160

Phe Ser Ile Arg Lys Glu Asn Leu Thr Glu Leu Ala Asn Ile Glu Ile  
 165 170 175

Leu Tyr Leu Gly Gln Asn Cys Tyr Tyr Arg Asn Pro Cys Tyr Val Ser  
 180 185 190

Tyr Ser Ile Glu Lys Asp Ala Phe Leu Asn Leu Thr Lys Leu Lys Val  
 195 200 205

Leu Ser Leu Lys Asp Asn Asn Val Thr Ala Val Pro Thr Val Leu Pro  
 210 215 220

Ser Thr Leu Thr Glu Leu Tyr Leu Tyr Asn Asn Met Ile Ala Lys Ile  
 225 230 235 240

Gln Glu Asp Asp Phe Asn Asn Leu Asn Gln Leu Gln Ile Leu Asp Leu  
 245 250 255

Ser Gly Asn Cys Pro Arg Cys Tyr Asn Ala Pro Phe Pro Cys Ala Pro  
 260 265 270

Cys Lys Asn Asn Ser Pro Leu Gln Ile Pro Val Asn Ala Phe Asp Ala  
 275 280 285

Leu Thr Glu Leu Lys Val Leu Arg Leu His Ser Asn Ser Leu Gln His  
 290 295 300

Val Pro Pro Arg Trp Phe Lys Asn Ile Asn Lys Leu Gln Glu Leu Asp  
 305 310 315 320

Leu Ser Gln Asn Phe Leu Ala Lys Glu Ile Gly Asp Ala Lys Phe Leu  
 325 330 335

His Phe Leu Pro Ser Leu Ile Gln Leu Asp Leu Ser Phe Asn Phe Glu  
 340 345 350

Leu Gln Val Tyr Arg Ala Ser Met Asn Leu Ser Gln Ala Phe Ser Ser  
 355 360 365

Leu Lys Ser Leu Lys Ile Leu Arg Ile Arg Gly Tyr Val Phe Lys Glu  
 370 375 380

Leu Lys Ser Phe Asn Leu Ser Pro Leu His Asn Leu Gln Asn Leu Glu  
 385 390 395 400

Val Leu Asp Leu Gly Thr Asn Phe Ile Lys Ile Ala Asn Leu Ser Met  
 405 410 415

Phe Lys Gln Phe Lys Arg Leu Lys Val Ile Asp Leu Ser Val Asn Lys  
 420 425 430

Ile Ser Pro Ser Gly Asp Ser Ser Glu Val Gly Phe Cys Ser Asn Ala  
 435 440 445

Arg Thr Ser Val Glu Ser Tyr Glu Pro Gln Val Leu Glu Gln Leu His  
 450 455 460

Tyr Phe Arg Tyr Asp Lys Tyr Ala Arg Ser Cys Arg Phe Lys Asn Lys  
 465 470 475 480

Glu Ala Ser Phe Met Ser Val Asn Glu Ser Cys Tyr Lys Tyr Gly Gln  
 485 490 495

Thr Leu Asp Leu Ser Lys Asn Ser Ile Phe Phe Val Lys Ser Ser Asp  
 500 505 510

Phe Gln His Leu Ser Phe Leu Lys Cys Leu Asn Leu Ser Gly Asn Leu  
 515 520 525

Ile Ser Gln Thr Leu Asn Gly Ser Glu Phe Gln Pro Leu Ala Glu Leu  
 530 535 540

Arg Tyr Leu Asp Phe Ser Asn Asn Arg Leu Asp Leu Leu His Ser Thr  
 545 550 555 560

Ala Phe Glu Glu Leu His Lys Leu Glu Val Leu Asp Ile Ser Ser Asn  
 565 570 575

Ser His Tyr Phe Gln Ser Glu Gly Ile Thr His Met Leu Asn Phe Thr  
 580 585 590

Lys Asn Leu Lys Val Leu Gln Lys Leu Met Met Asn Asp Asn Asp Ile  
 595 600 605

Ser Ser Ser Thr Ser Arg Thr Met Glu Ser Glu Ser Leu Arg Thr Leu  
 610 615 620

Glu Phe Arg Gly Asn His Leu Asp Val Leu Trp Arg Glu Gly Asp Asn  
 625 630 635 640

Arg Tyr Leu Gln Leu Phe Lys Asn Leu Leu Lys Leu Glu Glu Leu Asp  
 645 650 655

Ile Ser Lys Asn Ser Leu Ser Phe Leu Pro Ser Gly Val Phe Asp Gly  
 660 665 670

Met Pro Pro Asn Leu Lys Asn Leu Ser Leu Ala Lys Asn Gly Leu Lys  
 675 680 685

Ser Phe Ser Trp Lys Lys Leu Gln Cys Leu Lys Asn Leu Glu Thr Leu  
 690 695 700

Asp Leu Ser His Asn Gln Leu Thr Thr Val Pro Glu Arg Leu Ser Asn  
 705 710 715 720

Cys Ser Arg Ser Leu Lys Asn Leu Ile Leu Lys Asn Asn Gln Ile Arg  
 725 730 735

Ser Leu Thr Lys Tyr Phe Leu Gln Asp Ala Phe Gln Leu Arg Tyr Leu  
 740 745 750

Asp Leu Ser Ser Asn Lys Ile Gln Met Ile Gln Lys Thr Ser Phe Pro  
 755 760 765

Glu Asn Val Leu Asn Asn Leu Lys Met Leu Leu Leu His His Asn Arg  
 770 775 780

Phe Leu Cys Thr Cys Asp Ala Val Trp Phe Val Trp Trp Val Asn His  
 785 790 795 800

Thr Glu Val Thr Ile Pro Tyr Leu Ala Thr Asp Val Thr Cys Val Gly  
 805 810 815

Pro Gly Ala His Lys Gly Gln Ser Val Ile Ser Leu Asp Leu Tyr Thr  
 820 825 830

Cys Glu Leu Asp Leu Thr Asn Leu Ile Leu Phe Ser Leu Ser Ile Ser  
 835 840 845

Val Ser Leu Phe Leu Met Val Met Met Thr Ala Ser His Leu Tyr Phe  
 850 855 860

Trp Asp Val Trp Tyr Ile Tyr His Phe Cys Lys Ala Lys Ile Lys Gly  
 865 870 875 880

Tyr Gln Arg Leu Ile Ser Pro Asp Cys Cys Tyr Asp Ala Phe Ile Val  
 885 890 895

Tyr Asp Thr Lys Asp Pro Ala Val Thr Glu Trp Val Leu Ala Glu Leu  
 900 905 910

Val Ala Lys Leu Glu Asp Pro Arg Glu Lys His Phe Asn Leu Cys Leu  
 915 920 925

Glu Glu Arg Asp Trp Leu Pro Gly Gln Pro Val Leu Glu Asn Leu Ser  
 930 935 940

Gln Ser Ile Gln Leu Ser Lys Lys Thr Val Phe Val Met Thr Asp Lys  
 945 950 955 960

Tyr Ala Lys Thr Glu Asn Phe Lys Ile Ala Phe Tyr Leu Ser His Gln  
 965 970 975

Arg Leu Met Asp Glu Lys Val Asp Val Ile Ile Leu Ile Phe Leu Glu

	980		985		990
Lys Pro Phe Gln Lys Ser Lys Phe Leu Gln Leu Arg Lys Arg Leu Cys					
995		1000		1005	
Gly Ser Ser Val Leu Glu Trp Pro Thr Asn Pro Gln Ala His Pro					
1010		1015		1020	
Tyr Phe Trp Gln Cys Leu Lys Asn Ala Leu Ala Thr Asp Asn His					
1025		1030		1035	
Val Ala Tyr Ser Gln Val Phe Lys Glu Thr Val					
1040		1045			

<210> 25  
 <211> 5007  
 <212> DNA  
 <213> Homo sapiens

<400> 25  
 actccagata taggatcact ccatgccatc aagaaagttg atgctattgg gcccatctca 60  
 agctgatctt ggcacctctc atgctctgct ctcttcaacc agacctctac attccatttt 120  
 ggaagaagac taaaaatggg gttccaatg tggacactga agagacaaat tcttatcctt 180  
 tttaacataa tcctaatttc caaactcctt ggggctagat ggtttcctaa aactctgccc 240  
 tgtgatgtca ctctggatgt tccaaagaac catgtgatcg tggactgcac agacaagcat 300  
 ttgacagaaa ttcttgaggg tattcccacg aacaccacga acctcaccct caccattaac 360  
 cacataccag acatctcccc agcgtccttt cacagactgg accatctggg agagatcgat 420  
 ttcagatgca actgtgtacc tattccactg gggtcacaaa acaacatgtg catcaagagg 480  
 ctgcagatta aaccacagaag ctttagtgga ctcaattatt taaaatccct ttacctggat 540  
 ggaaaccagc tactagagat accgcagggc ctccgccta gcttacagct tctcagcctt 600  
 gaggccaaca acatcttttc catcagaaaa gagaatctaa cagaactggc caacatagaa 660  
 atactctacc tgggccaata ctgttattat cgaaatcctt gttatgtttc atattcaata 720  
 gagaaagatg ccttcctaaa cttgacaaaag ttaaaagtgc tctccctgaa agataacaat 780  
 gtcacagccg tcctactgt tttgccatct actttaacag aactatatct ctacaacaac 840  
 atgattgcaa aaatccaaga agatgatctt aataacctca accaattaca aattcttgac 900  
 ctaagtggaa attgccctcg ttgttataat gcccatttc cttgtgcgcc gtgtaaaaat 960  
 aattctcccc tacagatccc tgtaaatgct tttgatgcgc tgacagaatt aaaagtttta 1020

cgtctacaca gtaactctct tcagcatgtg cccccaagat ggtttaagaa catcaacaaa	1080
ctccaggaac tggatctgtc caaaaacttc ttggccaaag aaattgggga tgctaaattt	1140
ctgcattttc tccccagcct catccaattg gatctgtctt tcaattttga acttcaggtc	1200
tatcgtgcat ctatgaatct atcacaagca ttttcttcac tgaaaagcct gaaaattctg	1260
cggatcagag gatatgtctt taaagagttg aaaagcttta acctctcgcc attacataat	1320
cttcaaaatc ttgaagttct tgatcttggc actaacttta taaaaattgc taacctcagc	1380
atgtttaaac aatttaaaag actgaaagtc atagatcttt cagtgaataa aatatcacct	1440
tcaggagatt caagtgaagt tggcttctgc tcaaatgcc aaccttctgt agaaagtat	1500
gaacccagg tcttgaaca attacattat ttcagatatg ataagtatgc aaggagtgc	1560
agattcaaaa acaaagaggc ttctttcatg tctgttaatg aaagctgcta caagtatggg	1620
cagaccttgg atctaagtaa aaatagtata tttttgtca agtcctctga ttttcagcat	1680
ctttctttcc tcaaatgcct gaatctgtca ggaaatctca ttagccaaac tcttaatggc	1740
agtgaattcc aacctttagc agagctgaga tatttggact tctccaaca ccggttgat	1800
ttactccatt caacagcatt tgaagagctt caaaaactgg aagttctgga tataagcagt	1860
aatagccatt attttcaatc agaaggaatt actcatatgc taaactttac caagaaccta	1920
aaggttctgc agaaactgat gatgaacgac aatgacatct cttcctccac cagcaggacc	1980
atggagagtg agtctcttag aactctggaa ttcagaggaa atcacttaga tgttttatgg	2040
agagaaggty ataacagata cttacaatta ttcaagaatc tgctaaaatt agaggaaatta	2100
gacatctcta aaaattccct aagtttcttg cttctggag tttttgatgg tatgcctcca	2160
aatctaaaga atctctcttt ggccaaaat gggctcaaat ctttcagttg gaagaaactc	2220
cagtgtctaa agaacttgga aactttggac ctcagccaca accaactgac cactgtccct	2280
gagagattat ccaactgttc cagaagcctc aagaatctga ttcttaagaa taatcaaactc	2340
aggagtctga cgaagtattt tctacaagat gccttcagc tgcgatatct ggatctcagc	2400
tcaaataaaa tccagatgat ccaaaagacc agcttcccag aaaatgtcct caacaatctg	2460
aagatgttgc ttttgcatca taatcggtt ctgtgcacct gtgatgctgt gtggtttgtc	2520
tgggtgggtta accatacggg ggtgactatt ccttacctgg ccacagatgt gacttgtgtg	2580
gggccaggag cacacaaggg ccaaagtgtg atctccctgg atctgtacac ctgtgagtta	2640
gatctgacta acctgattct gttctcactt tccatatctg tatctctctt tctcatgggtg	2700
atgatgacag caagtcacct ctatttctgg gatgtgtggt atatttacca tttctgtaag	2760
gccaagataa aggggtatca gcgtctaata tcaccagact gttgctatga tgcttttatt	2820

gtgtatgaca	ctaaagaccc	agctgtgacc	gagtgggttt	tggctgagct	ggtggccaaa	2880
ctggaagacc	caagagagaa	acattttaat	ttatgtctcg	aggaaagga	ctggttacca	2940
gggcagccag	ttctggaaaa	cctttcccag	agcatacagc	ttagcaaaaa	gacagtgttt	3000
gtgatgacag	acaagtatgc	aaagactgaa	aattttaaga	tagcatttta	cttgtcccat	3060
cagaggctca	tggatgaaaa	agttgatgtg	attatcttga	tatttcttga	gaagcccttt	3120
cagaagtcca	agttcctcca	gctccggaaa	aggctctgtg	ggagttctgt	ccttgagtgg	3180
ccaacaaacc	cgcaagctca	cccatacttc	tggcagtgtc	taaagaacgc	cctggccaca	3240
gacaatcatg	tggcctatag	tcaggtgttc	aaggaaacgg	tctagccctt	ctttgcaaaa	3300
cacaactgcc	tagtttacca	aggagaggcc	tggctgttta	aattgttttc	atatatatca	3360
cacaaaaagc	gtgttttgaa	attcttcaag	aaatgagatt	gcccatattt	caggggagcc	3420
accaacgtct	gtcacaggag	ttggaaagat	ggggtttata	taatgcatca	agtcttcttt	3480
cttatctctc	tgtgtctcta	tttgcacttg	agtctctcac	ctcagctcct	gtaaaagagt	3540
ggcaagtaaa	aaacatgggg	ctctgattct	cctgtaattg	tgataattaa	atatacacac	3600
aatcatgaca	ttgagaagaa	ctgcatttct	acccttaaaa	agtactggta	tatacagaaa	3660
taggggttaa	aaaaactcaa	gctctctcta	tatgagacca	aaatgtacta	gagttagttt	3720
agtgaaataa	aaaaccagtc	agctggccgg	gcatgggtggc	tcatgcttgt	aatcccagca	3780
ctttgggagg	ccgaggcagg	tggatcacga	ggtcaggagt	ttgagaccag	tctggccaac	3840
atgggtgaaac	cccgtctgta	ctaaaaatac	aaaaattagc	tgggcgtggg	ggtgggtgcc	3900
tgtaatccca	gctacttggg	aggctgaggc	aggagaatcg	cttgaaccgc	ggaggtggag	3960
gtggcagtg	gccgagatca	cgccactgca	atgcagcccc	ggcaacagag	ctagactgtc	4020
tcaaaagaac	aaaaaaaaaa	aaacacaaaa	aaactcagtc	agcttcttaa	ccaattgctt	4080
ccgtgtcatc	cagggcccca	ttctgtgcag	attgagtgtg	ggcaccacac	agggtggtgc	4140
tgcttcagtg	cttcctgctc	tttttccttg	ggcctgcttc	tgggttccat	agggaacag	4200
taagaaagaa	agacacatcc	ttaccataaa	tgcataatgg	ccacctacaa	atagaaaaat	4260
atttaaataga	tctgccttta	tacaaagtga	tattctctac	ctttgataat	ttacctgctt	4320
aaatgttttt	atctgcactg	caaagtactg	tatccaaagt	aaaatttcct	catccaatat	4380
ctttcaaact	gttttggtta	ctaatgccat	atatttgtaa	gtatctgcac	acttgatata	4440
gcaacgtag	atgggtttga	tggttaaacc	taaaggagga	ctccaagagt	gtgtatttat	4500
ttatagtttt	atcagagatg	acaattattt	gaatgccaat	tatatggatt	cctttcatth	4560
tttgctggag	gatgggagaa	gaaaccaaag	tttatagacc	ttcacattga	gaaagcttca	4620

gttttgaact tcagctatca gattcaaaaa caacagaaag aaccaagaca ttcttaagat 4680  
gcctgtactt tcagctgggt ataaattcat gagttcaaag attgaaacct gaccaatttg 4740  
ctttatttca tggaagaagt gatctacaaa ggtgtttgtg ccatttgga aacagcgtgc 4800  
atgtgttcaa gccttagatt ggcgatgtcg tattttcctc acgtgtggca atgccaaagg 4860  
ctttacttta cctgtgagta cacactatat gaattatttc caacgtacat ttaatcaata 4920  
agggtcacaa attcccaaatt caatctctgg aataaataga gaggtaatta aattgctgga 4980  
gccaaactatt tcacaacttc tgtaagc 5007

<210> 26  
<211> 1050  
<212> PRT  
<213> Mus musculus

<400> 26

Met Val Phe Ser Met Trp Thr Arg Lys Arg Gln Ile Leu Ile Phe Leu  
1 5 10 15

Asn Met Leu Leu Val Ser Arg Val Phe Gly Phe Arg Trp Phe Pro Lys  
20 25 30

Thr Leu Pro Cys Glu Val Lys Val Asn Ile Pro Glu Ala His Val Ile  
35 40 45

Val Asp Cys Thr Asp Lys His Leu Thr Glu Ile Pro Glu Gly Ile Pro  
50 55 60

Thr Asn Thr Thr Asn Leu Thr Leu Thr Ile Asn His Ile Pro Ser Ile  
65 70 75 80

Ser Pro Asp Ser Phe Arg Arg Leu Asn His Leu Glu Glu Ile Asp Leu  
85 90 95

Arg Cys Asn Cys Val Pro Val Leu Leu Gly Ser Lys Ala Asn Val Cys  
100 105 110

Thr Lys Arg Leu Gln Ile Arg Pro Gly Ser Phe Ser Gly Leu Ser Asp  
115 120 125

Leu Lys Ala Leu Tyr Leu Asp Gly Asn Gln Leu Leu Glu Ile Pro Gln  
130 135 140

Asp Leu Pro Ser Ser Leu His Leu Leu Ser Leu Glu Ala Asn Asn Ile  
 145 150 155 160

Phe Ser Ile Thr Lys Glu Asn Leu Thr Glu Leu Val Asn Ile Glu Thr  
 165 170 175

Leu Tyr Leu Gly Gln Asn Cys Tyr Tyr Arg Asn Pro Cys Asn Val Ser  
 180 185 190

Tyr Ser Ile Glu Lys Asp Ala Phe Leu Val Met Arg Asn Leu Lys Val  
 195 200 205

Leu Ser Leu Lys Asp Asn Asn Val Thr Ala Val Pro Thr Thr Leu Pro  
 210 215 220

Pro Asn Leu Leu Glu Leu Tyr Leu Tyr Asn Asn Ile Ile Lys Lys Ile  
 225 230 235 240

Gln Glu Asn Asp Phe Asn Asn Leu Asn Glu Leu Gln Val Leu Asp Leu  
 245 250 255

260

265

270

Cys Glu Asn Asn Ser Pro Leu Gln Ile His Asp Asn Ala Phe Asn Ser  
 275 280 285

Leu Thr Glu Leu Lys Val Leu Arg Leu His Ser Asn Ser Leu Gln His  
 290 295 300

Val Pro Pro Thr Trp Phe Lys Asn Met Arg Asn Leu Gln Glu Leu Asp  
 305 310 315 320

Leu Ser Gln Asn Tyr Leu Ala Arg Glu Ile Glu Glu Ala Lys Phe Leu  
 325 330 335

His Phe Leu Pro Asn Leu Val Glu Leu Asp Phe Ser Phe Asn Tyr Glu  
 340 345 350

Leu Gln Val Tyr His Ala Ser Ile Thr Leu Pro His Ser Leu Ser Ser  
 355 360 365

Leu Glu Asn Leu Lys Ile Leu Arg Val Lys Gly Tyr Val Phe Lys Glu  
 370 375 380

Leu Lys Asn Ser Ser Leu Ser Val Leu His Lys Leu Pro Arg Leu Glu  
385 390 395 400

Val Leu Asp Leu Gly Thr Asn Phe Ile Lys Ile Ala Asp Leu Asn Ile  
405 410 415

Phe Lys His Phe Glu Asn Leu Lys Leu Ile Asp Leu Ser Val Asn Lys  
420 425 430

Ile Ser Pro Ser Glu Glu Ser Arg Glu Val Gly Phe Cys Pro Asn Ala  
435 440 445

Gln Thr Ser Val Asp Arg His Gly Pro Gln Val Leu Glu Ala Leu His  
450 455 460

Tyr Phe Arg Tyr Asp Glu Tyr Ala Arg Ser Cys Arg Phe Lys Asn Lys  
465 470 475 480

Glu Pro Pro Ser Phe Leu Pro Leu Asn Ala Asp Cys His Ile Tyr Gly  
485 490 495

Gln Thr Leu Asp Leu Ser Arg Asn Asn Ile Phe Phe Ile Lys Pro Ser  
500 505 510

Asp Phe Gln His Leu Ser Phe Leu Lys Cys Leu Asn Leu Ser Gly Asn  
515 520 525

Thr Ile Gly Gln Thr Leu Asn Gly Ser Glu Leu Trp Pro Leu Arg Glu  
530 535 540

Leu Arg Tyr Leu Asp Phe Ser Asn Asn Arg Leu Asp Leu Leu Tyr Ser  
545 550 555 560

Thr Ala Phe Glu Glu Leu Gln Ser Leu Glu Val Leu Asp Leu Ser Ser  
565 570 575

Asn Ser His Tyr Phe Gln Ala Glu Gly Ile Thr His Met Leu Asn Phe  
580 585 590

Thr Lys Lys Leu Arg Leu Leu Asp Lys Leu Met Met Asn Asp Asn Asp  
595 600 605

Ile Ser Thr Ser Ala Ser Arg Thr Met Glu Ser Asp Ser Leu Arg Ile  
610 615 620

Leu Glu Phe Arg Gly Asn His Leu Asp Val Leu Trp Arg Ala Gly Asp  
625 630 635 640

Asn Arg Tyr Leu Asp Phe Phe Lys Asn Leu Phe Asn Leu Glu Val Leu  
645 650 655

Asp Ile Ser Arg Asn Ser Leu Asn Ser Leu Pro Pro Glu Val Phe Glu  
660 665 670

Gly Met Pro Pro Asn Leu Lys Asn Leu Ser Leu Ala Lys Asn Gly Leu  
675 680 685

Lys Ser Phe Phe Trp Asp Arg Leu Gln Leu Leu Lys His Leu Glu Ile  
690 695 700

Leu Asp Leu Ser His Asn Gln Leu Thr Lys Val Pro Glu Arg Leu Ala  
705 710 715 720

Asn Cys Ser Lys Ser Leu Thr Thr Leu Ile Leu Lys His Asn Gln Ile  
725 730 735

Arg Gln Leu Thr Lys Tyr Phe Leu Glu Asp Ala Leu Gln Leu Arg Tyr  
740 745 750

Leu Asp Ile Ser Ser Asn Lys Ile Gln Val Ile Gln Lys Thr Ser Phe  
755 760 765

770

775

780

Arg Phe Leu Cys Asn Cys Asp Ala Val Trp Phe Val Trp Trp Val Asn  
785 790 795 800

His Thr Asp Val Thr Ile Pro Tyr Leu Ala Thr Asp Val Thr Cys Val  
805 810 815

Gly Pro Gly Ala His Lys Gly Gln Ser Val Ile Ser Leu Asp Leu Tyr  
820 825 830

Thr Cys Glu Leu Asp Leu Thr Asn Leu Ile Leu Phe Ser Val Ser Ile  
835 840 845

Ser Ser Val Leu Phe Leu Met Val Val Met Thr Thr Ser His Leu Phe  
850 855 860

Phe Trp Asp Met Trp Tyr Ile Tyr Tyr Phe Trp Lys Ala Lys Ile Lys  
865 870 875 880

Gly Tyr Gln His Leu Gln Ser Met Glu Ser Cys Tyr Asp Ala Phe Ile  
885 890 895

Val Tyr Asp Thr Lys Asn Ser Ala Val Thr Glu Trp Val Leu Gln Glu  
900 905 910

Leu Val Ala Lys Leu Glu Asp Pro Arg Glu Lys His Phe Asn Leu Cys  
915 920 925

Leu Glu Glu Arg Asp Trp Leu Pro Gly Gln Pro Val Leu Glu Asn Leu  
930 935 940

Ser Gln Ser Ile Gln Leu Ser Lys Lys Thr Val Phe Val Met Thr Gln  
945 950 955 960

Lys Tyr Ala Lys Thr Glu Ser Phe Lys Met Ala Phe Tyr Leu Ser His  
965 970 975

Gln Arg Leu Leu Asp Glu Lys Val Asp Val Ile Ile Leu Ile Phe Leu  
980 985 990

Glu Lys Pro Leu Gln Lys Ser Lys Phe Leu Gln Leu Arg Lys Arg Leu  
995 1000 1005

Cys Arg Ser Ser Val Leu Glu Trp Pro Ala Asn Pro Gln Ala His  
1010 1015 1020

Pro Tyr Phe Trp Gln Cys Leu Lys Asn Ala Leu Thr Thr Asp Asn  
1025 1030 1035

His Val Ala Tyr Ser Gln Met Phe Lys Glu Thr Val  
1040 1045 1050

<210> 27  
<211> 3243  
<212> DNA  
<213> Mus musculus

<400> 27  
attctcctcc accagacctc ttgattccat tttgaaagaa aactgaaaat ggtgttttcg 60  
atgtggacac ggaagagaca aattttgatc tttttaaata tgctcttagt ttctagagtc 120

tttgggtttc gatggtttcc taaaactcta ccttgtgaag ttaaagtaaa tatcccagag	180
gcccattgtga tcgtggactg cacagacaag catttgacag aaatccctga gggcattccc	240
actaacacca ccaatcttac ccttaccatc aaccacatac caagcatctc tccagattcc	300
ttccgtaggc tgaaccatct ggaagaaatc gatttaagat gcaatttgtgt acctgttcta	360
ctgggggtcca aagccaatgt gtgtaccaag aggctgcaga ttagacctgg aagctttagt	420
ggactctctg acttaaaagc cctttacctg gatggaaacc aacttctgga gataccacag	480
gatctgccat ccagcttaca tcttctgagc cttgaggcta acaacatctt ctccatcacg	540
aaggagaatc taacagaact ggtcaacatt gaaacactct acctgggtca aaactgttat	600
tatcgaaatc cttgcaatgt ttctattctt attgaaaaag atgctttcct agttatgaga	660
aatttgaagg ttctctcact aaaagataac aatgtcacag ctgtccccac cactttgcc	720
cctaatttac tagagctcta tctttataac aatatcatta agaaaatcca agaaaatgat	780
tttaataacc tcaatgagtt gcaagttctt gacctaatg gaaattgccc tcgatgttat	840
aatgtcccat atccgtgtac accgtgtgaa aataattccc ccttacagat ccatgacaat	900
gctttcaatt cattgacaga attaaaagtt ttacgtttac acagtaattc tcttcagcat	960
gtgcccccaa catggtttaa aaacatgaga aacctccagg aactagacct ctccccaaac	1020
tacttggcca gagaaattga ggaggccaaa tttttgcatt ttcttcccaa ccttgttgag	1080
ttggattttt ctttcaatta tgagctgcag gtctaccatg catctataac tttaccacat	1140
tcactctctt cattggaaaa cttgaaaatt ctgcgtgtca aggggtatgt ctttaaagag	1200
ctgaaaaact ccagtcttct tgtattgcac aagcttccca ggctggaagt tcttgacctt	1260
ctcatagacc tttcagtga taagatatct ccttcagaag agtcaagaga agttggcttt	1380
tgctctaattg ctcaaacttc tgtagaccgt catgggcccc aggtccttga ggccttacac	1440
tatttccgat acgatgaata tgcacggagc tgcaggttca aaaacaaaga gccaccttct	1500
ttcttgcctt tgaatgcaga ctgccacata tatgggcaga ccttagactt aagtagaaat	1560
aacatatttt ttattaaacc ttctgatttt cagcatcttt cattcctcaa atgcctcaac	1620
ttatcaggaa acaccattgg ccaaactctt aatggcagtg aactctggcc gttgagagag	1680
ttgcggtact tagacttctc caacaaccgg cttgatttac tctactcaac agcctttgaa	1740
gagctccaga gtcttgaagt tctggatcta agtagtaaca gccactattt tcaagcagaa	1800
ggaattactc acatgctaaa ctttaccag aaattacggc ttctggacaa actcatgatg	1860
aatgataatg acatctctac ttcggccagc aggacctgg aaagtgactc tcttcgaatt	1920

```

ctggagttca gaggcaacca ttagatggtt ctatggagag cgggtgataa cagatacttg 1980
gacttcttca agaatttggt caatttagag gtattagata tctccagaaa ttccctgaat 2040
tccttgctc ctgagggttt tgagggtatg ccgccaatc taaagaatct ctcttggtcc 2100
aaaaatgggc tcaaattctt cttttgggac agactccagt tactgaagca tttggaaatt 2160
ttggacctca gccataacca gctgacaaaa gtacctgaga gattggccaa ctgttccaaa 2220
agtctcacia cactgattct taagcataat caaatcaggc aattgacaaa atattttcta 2280
gaagatgctt tgcaattgcg ctatctagac atcagttcaa ataaaatcca ggtcattcag 2340
aagactagct tcccagaaaa tgcctcaac aatctggaga tgttggtttt acatcacaat 2400
cgctttcttt gcaactgtga tgctgtgtgg tttgtctggt gggttaacca tacagatggt 2460
actattccat acctggccac tgatgtgact tgtgtaggtc caggagcaca caaagggtcaa 2520
agtgtcatat cccttgatct gtatacgtgt gagttagatc tcacaaacct gattctgttc 2580
tcagtttcca tatcatcagt cctctttctt atggtagtta tgacaacaag tcacctcttt 2640
ttctgggata tgtggtacat ttattatttt tggaaagcaa agataaagggt gtatcagcat 2700
ctgcaatcca tggagtcttg ttatgatgct tttattgtgt atgacactaa aaactcagct 2760
gtgacagaat ggggttttgca ggagctggtg gcaaaattgg aagatccaag agaaaaacac 2820
ttcaatttgt gtctagaaga aagagactgg ctaccaggac agccagttct agaaaacctt 2880
tcccagagca tacagctcag caaaaagaca gtgtttgtga tgacacagaa atatgctaag 2940
actgagagtt ttaagatggc attttatttg tctcatcaga ggctcctgga tgaaaaagtg 3000
gatgtgatta tcttgatatt cttggaaaag cctcttcaga agtctaagtt tcttcagctc 3060
aggaagagac tctgcaggag ctctgtcctt gagtggcctg caaatccaca ggctcaccca 3120
tacttctggc agtgcctgaa aaatgcctg accacagaca atcatgtggc ttatagtcaa 3180
atgttcaagg aaacagtcta gctctctgaa gaatgtcacc acctaggaca tgcttgaat 3240
cga 3243

```

```

<210> 28
<211> 1041
<212> PRT
<213> Homo sapiens

```

```

<400> 28

```

```

Met Glu Asn Met Phe Leu Gln Ser Ser Met Leu Thr Cys Ile Phe Leu
1           5           10           15

```

```

Leu Ile Ser Gly Ser Cys Glu Leu Cys Ala Glu Glu Asn Phe Ser Arg

```

20	25	30	
Ser Tyr Pro Cys Asp Glu Lys	Lys Gln Asn Asp Ser Val Ile Ala Glu		
35	40	45	
Cys Ser Asn Arg Arg Leu Gln Glu Val Pro Gln Thr Val Gly Lys Tyr			
50	55	60	
Val Thr Glu Leu Asp Leu Ser Asp Asn Phe Ile Thr His Ile Thr Asn			
65	70	75	80
Glu Ser Phe Gln Gly Leu Gln Asn Leu Thr Lys Ile Asn Leu Asn His			
85	90	95	
Asn Pro Asn Val Gln His Gln Asn Gly Asn Pro Gly Ile Gln Ser Asn			
100	105	110	
Gly Leu Asn Ile Thr Asp Gly Ala Phe Leu Asn Leu Lys Asn Leu Arg			
115	120	125	
Glu Leu Leu Leu Glu Asp Asn Gln Leu Pro Gln Ile Pro Ser Gly Leu			
130	135	140	
Pro Glu Ser Leu Thr Glu Leu Ser Leu Ile Gln Asn Asn Ile Tyr Asn			
145	150	155	160
Ile Thr Lys Glu Gly Ile Ser Arg Leu Ile Asn Leu Lys Asn Leu Tyr			
165	170	175	
Leu Ala Trp Asn Cys Tyr Phe Asn Lys Val Cys Glu Lys Thr Asn Ile			
180	185	190	
Glu Asp Gly Val Phe Glu Thr Leu Thr Asn Leu Glu Leu Leu Ser Leu			
195	200	205	
Ser Phe Asn Ser Leu Ser His Val Pro Pro Lys Leu Pro Ser Ser Leu			
210	215	220	
Arg Lys Leu Phe Leu Ser Asn Thr Gln Ile Lys Tyr Ile Ser Glu Glu			
225	230	235	240
Asp Phe Lys Gly Leu Ile Asn Leu Thr Leu Leu Asp Leu Ser Gly Asn			
245	250	255	
Cys Pro Arg Cys Phe Asn Ala Pro Phe Pro Cys Val Pro Cys Asp Gly			

260	265	270
Gly Ala Ser Ile Asn Ile Asp Arg Phe Ala Phe Gln Asn Leu Thr Gln 275 280 285		
Leu Arg Tyr Leu Asn Leu Ser Ser Thr Ser Leu Arg Lys Ile Asn Ala 290 295 300		
Ala Trp Phe Lys Asn Met Pro His Leu Lys Val Leu Asp Leu Glu Phe 305 310 315 320		
Asn Tyr Leu Val Gly Glu Ile Ala Ser Gly Ala Phe Leu Thr Met Leu 325 330 335		
Pro Arg Leu Glu Ile Leu Asp Leu Ser Phe Asn Tyr Ile Lys Gly Ser 340 345 350		
Tyr Pro Gln His Ile Asn Ile Ser Arg Asn Phe Ser Lys Leu Leu Ser 355 360 365		
Leu Arg Ala Leu His Leu Arg Gly Tyr Val Phe Gln Glu Leu Arg Glu 370 375 380		
Asp Asp Phe Gln Pro Leu Met Gln Leu Pro Asn Leu Ser Thr Ile Asn 385 390 395 400		
Leu Gly Ile Asn Phe Ile Lys Gln Ile Asp Phe Lys Leu Phe Gln Asn 405 410 415		
Phe Ser Asn Leu Glu Ile Ile Tyr Leu Ser Glu Asn Arg Ile Ser Pro 420 425 430		
Leu Val Lys Asp Thr Arg Gln Ser Tyr Ala Asn Ser Ser Ser Phe Gln 435 440 445		
Arg His Ile Arg Lys Arg Arg Ser Thr Asp Phe Glu Phe Asp Pro His 450 455 460		
Ser Asn Phe Tyr His Phe Thr Arg Pro Leu Ile Lys Pro Gln Cys Ala 465 470 475 480		
Ala Tyr Gly Lys Ala Leu Asp Leu Ser Leu Asn Ser Ile Phe Phe Ile 485 490 495		

Gly Pro Asn Gln Phe Glu Asn Leu Pro Asp Ile Ala Cys Leu Asn Leu  
 500 505 510

Ser Ala Asn Ser Asn Ala Gln Val Leu Ser Gly Thr Glu Phe Ser Ala  
 515 520 525

Ile Pro His Val Lys Tyr Leu Asp Leu Thr Asn Asn Arg Leu Asp Phe  
 530 535 540

Asp Asn Ala Ser Ala Leu Thr Glu Leu Ser Asp Leu Glu Val Leu Asp  
 545 550 555 560

Leu Ser Tyr Asn Ser His Tyr Phe Arg Ile Ala Gly Val Thr His His  
 565 570 575

Leu Glu Phe Ile Gln Asn Phe Thr Asn Leu Lys Val Leu Asn Leu Ser  
 580 585 590

His Asn Asn Ile Tyr Thr Leu Thr Asp Lys Tyr Asn Leu Glu Ser Lys  
 595 600 605

Ser Leu Val Glu Leu Val Phe Ser Gly Asn Arg Leu Asp Ile Leu Trp  
 610 615 620

Asn Asp Asp Asp Asn Arg Tyr Ile Ser Ile Phe Lys Gly Leu Lys Asn  
 625 630 635 640

Leu Thr Arg Leu Asp Leu Ser Leu Asn Arg Leu Lys His Ile Pro Asn  
 645 650 655

Glu Ala Phe Leu Asn Leu Pro Ala Ser Leu Thr Glu Leu His Ile Asn  
 660 665 670

Asp Asn Met Leu Lys Phe Phe Asn Trp Thr Leu Leu Gln Gln Phe Pro  
 675 680 685

Arg Leu Glu Leu Leu Asp Leu Arg Gly Asn Lys Leu Leu Phe Leu Thr  
 690 695 700

Asp Ser Leu Ser Asp Phe Thr Ser Ser Leu Arg Thr Leu Leu Leu Ser  
 705 710 715 720

His Asn Arg Ile Ser His Leu Pro Ser Gly Phe Leu Ser Glu Val Ser  
 725 730 735

Ser Leu Lys His Leu Asp Leu Ser Ser Asn Leu Leu Lys Thr Ile Asn  
 740 745 750

Lys Ser Ala Leu Glu Thr Lys Thr Thr Thr Lys Leu Ser Met Leu Glu  
 755 760 765

Leu His Gly Asn Pro Phe Glu Cys Thr Cys Asp Ile Gly Asp Phe Arg  
 770 775 780

Arg Trp Met Asp Glu His Leu Asn Val Lys Ile Pro Arg Leu Val Asp  
 785 790 795 800

Val Ile Cys Ala Ser Pro Gly Asp Gln Arg Gly Lys Ser Ile Val Ser  
 805 810 815

Leu Glu Leu Thr Thr Cys Val Ser Asp Val Thr Ala Val Ile Leu Phe  
 820 825 830

Phe Phe Thr Phe Phe Ile Thr Thr Met Val Met Leu Ala Ala Leu Ala  
 835 840 845

His His Leu Phe Tyr Trp Asp Val Trp Phe Ile Tyr Asn Val Cys Leu  
 850 855 860

Ala Lys Val Lys Gly Tyr Arg Ser Leu Ser Thr Ser Gln Thr Phe Tyr  
 865 870 875 880

Asp Ala Tyr Ile Ser Tyr Asp Thr Lys Asp Ala Ser Val Thr Asp Trp  
 885 890 895

Val Ile Asn Glu Leu Arg Tyr His Leu Glu Glu Ser Arg Asp Lys Asn  
 900 905 910

Val Leu Leu Cys Leu Glu Glu Arg Asp Trp Asp Pro Gly Leu Ala Ile  
 915 920 925

Ile Asp Asn Leu Met Gln Ser Ile Asn Gln Ser Lys Lys Thr Val Phe  
 930 935 940

Val Leu Thr Lys Lys Tyr Ala Lys Ser Trp Asn Phe Lys Thr Ala Phe  
 945 950 955 960

Tyr Leu Ala Leu Gln Arg Leu Met Asp Glu Asn Met Asp Val Ile Ile  
 965 970 975

Phe Ile Leu Leu Glu Pro Val Leu Gln His Ser Gln Tyr Leu Arg Leu  
                   980                                  985                                  990

Arg Gln Arg Ile Cys Lys Ser Ser Ile Leu Gln Trp Pro Asp Asn Pro  
           995                                  1000                                  1005

Lys Ala Glu Gly Leu Phe Trp Gln Thr Leu Arg Asn Val Val Leu  
       1010                                  1015                                  1020

Thr Glu Asn Asp Ser Arg Tyr Asn Asn Met Tyr Val Asp Ser Ile  
       1025                                  1030                                  1035

Lys Gln Tyr  
       1040

<210> 29  
 <211> 3311  
 <212> DNA  
 <213> Homo sapiens

<400> 29  
 ttctgcgctg ctgcaagtta cggaatgaaa aattagaaca acagaaacat ggaaaacatg 60  
 ttccttcagt cgtcaatgct gacctgcatt ttcctgctaa tatctgggtc ctgtgagtta 120  
 tgcgccgaag aaaatttttc tagaagctat ccttgatgatg agaaaaagca aaatgactca 180  
 gttattgcag agtgcagcaa tcgtcgacta caggaagtgc cccaaacggt gggcaaatat 240  
 gtgacagaac tagacctgtc tgataatttc atcacacaca taacgaatga atcatttcaa 300  
 gggctgcaaa atctcactaa aataaatcta aaccacaacc ccaatgtaca gcaccagaac 360  
 ggaaatcccg gtatacaatc aaatggcttg aatatcacag acggggcatt cctcaaccta 420  
 aaaaacctaa gggagttact gcttgaagac aaccagttac ccaaataacc ctctggtttg 480  
 ccagagtctt tgacagaact tagtctaatt caaaacaata tataacaacat aactaaagag 540  
 ggcatttcaa gacttataaa cttgaaaaat ctctatttgg cctggaactg ctatttttaac 600  
 aaagtttgcg agaaaactaa catagaagat ggagtatttg aaacgctgac aaatttgagg 660  
 ttgctatcac tatctttcaa ttctctttca cacgtgccac ccaaactgcc aagctcccta 720  
 cgcaaacttt ttctgagcaa caccagatc aaatacatta gtgaagaaga tttcaaggga 780  
 ttgataaatt taacattact agatttaagc gggaactgtc cgaggtgctt caatgcccc 840  
 tttccatgcg tgcttgtga tgggtggtgct tcaattaata tagatcggtt tgcttttcaa 900  
 aacttgaccc aacttcgata cctaaacctc tctagcactt ccctcaggaa gattaatgct 960  
 gcctggttta aaaatatgcc tcactgaag gtgctggatc ttgaattcaa ctatttagtg 1020

ggagaaatag cctctggggc atttttaacg atgctgcccc gcttagaaat acttgacttg	1080
tcttttaact atataaaggg gagttatcca cagcatatta atatttccag aaacttctct	1140
aaacttttgt ctctacgggc attgcattta agaggttatg tgttccagga actcagagaa	1200
gatgatttcc agccctgat gcagcttcca aacttatcga ctatcaactt ggggtattaat	1260
tttattaagc aaatcgattt caaacttttc caaaatttct ccaatctgga aattatttac	1320
ttgtcagaaa acagaatatc accgttggtg aaagataccc ggcagagtta tgcaaatagt	1380
tcctcttttc aacgtcatat ccggaaacga cgctcaacag attttgagtt tgaccacat	1440
tcgaactttt atcatttcac ccgtccttta ataaagccac aatgtgctgc ttatggaaaa	1500
gccttagatt taagcctcaa cagtattttc ttcattgggc caaaccaatt tgaaaatctt	1560
cctgacattg cctgtttaaa tctgtctgca aatagcaatg ctcaagtgtt aagtggaact	1620
gaattttcag ccattcctca tgtcaaatat ttggatttga caaacaatag actagacttt	1680
gataatgcta gtgctcttac tgaattgtcc gacttggaag ttctagatct cagctataat	1740
tcacactatt tcagaatagc aggcgtaaca catcatctag aatttattca aaatttcaca	1800
aatctaaaag ttttaaactt gagccacaac aacatttata ctttaacaga taagtataac	1860
ctggaaagca agtccttggg agaattagtt ttcagtggca atcgcttga cattttgtgg	1920
aatgatgatg acaacaggta tatctccatt ttcaaaggtc tcaagaatct gacacgtctg	1980
gatttatccc ttaataggct gaagcacatc ccaaagaag cattccttaa tttgccagcg	2040
agtctcactg aactacatat aaatgataat atgttaaagt tttttaactg gacattactc	2100
cagcagttcc ctgctctcga gttgcttgac ttacgtggaa acaaactact ctttttaact	2160
gatagcctat ctgactttac atcttccctt cggacactgc tgctgagtca taacaggatt	2220
tcccacctac cctctggctt tctttctgaa gtcagtagtc tgaagcacct cgatttaagt	2280
tccaatctgc taaaaacaat caacaaatcc gcacttgaaa ctaagaccac caccaaatta	2340
tctatgttgg aactacacgg aaaccctttt gaatgcacct gtgacattgg agatttccga	2400
agatggatgg atgaacatct gaatgtcaaa attcccagac tggtagatgt catttgtgcc	2460
agtcttgggg atcaaagagg gaagagtatt gtgagtctgg agctgacaac ttgtgtttca	2520
gatgtcactg cagtgatatt atttttcttc acgttcttta tcaccacat gggtatgttg	2580
gctgccctgg ctaccattt gttttactgg gatgtttggg ttatatataa tgtgtgttta	2640
gctaaggtaa aaggctacag gtctctttcc acatcccaaa ctttctatga tgcttacatt	2700
tcttatgaca ccaaagatgc ctctgttact gactgggtga taaatgagct gcgctaccac	2760

```

cttgaagaga gccgagacaa aaacgttctc ctttgtctag aggagagggg ttgggacccg 2820
ggattggcca tcatcgacaa cctcatgcag agcatcaacc aaagcaagaa aacagtattt 2880
gttttaacca aaaaatatgc aaaaagctgg aacttttaaaa cagcttttta cttggctttg 2940
cagaggctaa tggatgagaa catggatgtg attatatatta tcctgctgga gccagtgtta 3000
cagcattctc agtatttgag gctacggcag cggatctgta agagctccat cctccagtgg 3060
cctgacaacc cgaaggcaga aggcttgttt tggcaaactc tgagaaatgt ggtcttgact 3120
gaaaatgatt cacggtataa caatatgtat gtcgattcca ttaagcaata ctaactgacg 3180
ttaagtcatg atttcgcgcc ataataaaga tgcaaaggaa tgacatttct gtattagtta 3240
tctattgcta tgtaacaaat tatcccaaaa cttagtgggt taaaacaaca catttgctgg 3300
cccacagttt t 3311

```

```

<210> 30
<211> 1059
<212> PRT
<213> Homo sapiens

```

```

<400> 30

```

```

Met Lys Glu Ser Ser Leu Gln Asn Ser Ser Cys Ser Leu Gly Lys Glu
1          5          10          15

```

```

Thr Lys Lys Glu Asn Met Phe Leu Gln Ser Ser Met Leu Thr Cys Ile
20          25          30

```

```

Phe Leu Leu Ile Ser Gly Ser Cys Glu Leu Cys Ala Glu Glu Asn Phe
35          40          45

```

```

Ser Arg Ser Tyr Pro Cys Asp Glu Lys Lys Gln Asn Asp Ser Val Ile
50          55          60

```

```

Ala Glu Cys Ser Asn Arg Arg Leu Gln Glu Val Pro Gln Thr Val Gly
65          70          75          80

```

```

Lys Tyr Val Thr Glu Leu Asp Leu Ser Asp Asn Phe Ile Thr His Ile
85          90          95

```

```

Thr Asn Glu Ser Phe Gln Gly Leu Gln Asn Leu Thr Lys Ile Asn Leu
100         105         110

```

```

Asn His Asn Pro Asn Val Gln His Gln Asn Gly Asn Pro Gly Ile Gln
115         120         125

```

Ser Asn Gly Leu Asn Ile Thr Asp Gly Ala Phe Leu Asn Leu Lys Asn  
 130 135 140

Leu Arg Glu Leu Leu Leu Glu Asp Asn Gln Leu Pro Gln Ile Pro Ser  
 145 150 155 160

Gly Leu Pro Glu Ser Leu Thr Glu Leu Ser Leu Ile Gln Asn Asn Ile  
 165 170 175

Tyr Asn Ile Thr Lys Glu Gly Ile Ser Arg Leu Ile Asn Leu Lys Asn  
 180 185 190

Leu Tyr Leu Ala Trp Asn Cys Tyr Phe Asn Lys Val Cys Glu Lys Thr  
 195 200 205

Asn Ile Glu Asp Gly Val Phe Glu Thr Leu Thr Asn Leu Glu Leu Leu  
 210 215 220

Ser Leu Ser Phe Asn Ser Leu Ser His Val Ser Pro Lys Leu Pro Ser  
 225 230 235 240

Ser Leu Arg Lys Leu Phe Leu Ser Asn Thr Gln Ile Lys Tyr Ile Ser  
 245 250 255

Glu Glu Asp Phe Lys Gly Leu Ile Asn Leu Thr Leu Leu Asp Leu Ser  
 260 265 270

Gly Asn Cys Pro Arg Cys Phe Asn Ala Pro Phe Pro Cys Val Pro Cys  
 275 280 285

Asp Gly Gly Ala Ser Ile Asn Ile Asp Arg Phe Ala Phe Gln Asn Leu  
 290 295 300

Thr Gln Leu Arg Tyr Leu Asn Leu Ser Ser Thr Ser Leu Arg Lys Ile  
 305 310 315 320

Asn Ala Ala Trp Phe Lys Asn Met Pro His Leu Lys Val Leu Asp Leu  
 325 330 335

Glu Phe Asn Tyr Leu Val Gly Glu Ile Ala Ser Gly Ala Phe Leu Thr  
 340 345 350

Met Leu Pro Arg Leu Glu Ile Leu Asp Leu Ser Phe Asn Tyr Ile Lys  
 355 360 365

Gly Ser Tyr Pro Gln His Ile Asn Ile Ser Arg Asn Phe Ser Lys Pro  
 370 375 380  
 Leu Ser Leu Arg Ala Leu His Leu Arg Gly Tyr Val Phe Gln Glu Leu  
 385 390 395 400  
 Arg Glu Asp Asp Phe Gln Pro Leu Met Gln Leu Pro Asn Leu Ser Thr  
 405 410 415  
 Ile Asn Leu Gly Ile Asn Phe Ile Lys Gln Ile Asp Phe Lys Leu Phe  
 420 425 430  
 Gln Asn Phe Ser Asn Leu Glu Ile Ile Tyr Leu Ser Glu Asn Arg Ile  
 435 440 445  
 Ser Pro Leu Val Lys Asp Thr Arg Gln Ser Tyr Ala Asn Ser Ser Ser  
 450 455 460  
 Phe Gln Arg His Ile Arg Lys Arg Arg Ser Thr Asp Phe Glu Phe Asp  
 465 470 475 480  
 Pro His Ser Asn Phe Tyr His Phe Thr Arg Pro Leu Ile Lys Pro Gln  
 485 490 495  
 Cys Ala Ala Tyr Gly Lys Ala Leu Asp Leu Ser Leu Asn Ser Ile Phe  
 500 505 510  
 Phe Ile Gly Pro Asn Gln Phe Glu Asn Leu Pro Asp Ile Ala Cys Leu  
 515 520 525  
 Asn Leu Ser Ala Asn Ser Asn Ala Gln Val Leu Ser Gly Thr Glu Phe  
 530 535 540  
 Ser Ala Ile Pro His Val Lys Tyr Leu Asp Leu Thr Asn Asn Arg Leu  
 545 550 555 560  
 Asp Phe Asp Asn Ala Ser Ala Leu Thr Glu Leu Ser Asp Leu Glu Val  
 565 570 575  
 Leu Asp Leu Ser Tyr Asn Ser His Tyr Phe Arg Ile Ala Gly Val Thr  
 580 585 590  
 His His Leu Glu Phe Ile Gln Asn Phe Thr Asn Leu Lys Val Leu Asn  
 595 600 605

Leu Ser His Asn Asn Ile Tyr Thr Leu Thr Asp Lys Tyr Asn Leu Glu  
610 615 620

Ser Lys Ser Leu Val Glu Leu Val Phe Ser Gly Asn Arg Leu Asp Ile  
625 630 635 640

Leu Trp Asn Asp Asp Asp Asn Arg Tyr Ile Ser Ile Phe Lys Gly Leu  
645 650 655

Lys Asn Leu Thr Arg Leu Asp Leu Ser Leu Asn Arg Leu Lys His Ile  
660 665 670

Pro Asn Glu Ala Phe Leu Asn Leu Pro Ala Ser Leu Thr Glu Leu His  
675 680 685

Ile Asn Asp Asn Met Leu Lys Phe Phe Asn Trp Thr Leu Leu Gln Gln  
690 695 700

Phe Pro Arg Leu Glu Leu Leu Asp Leu Arg Gly Asn Lys Leu Leu Phe  
705 710 715 720

Leu Thr Asp Ser Leu Ser Asp Phe Thr Ser Ser Leu Arg Thr Leu Leu  
725 730 735

Leu Ser His Asn Arg Ile Ser His Leu Pro Ser Gly Phe Leu Ser Glu  
740 745 750

Val Ser Ser Leu Lys His Leu Asp Leu Ser Ser Asn Leu Leu Lys Thr  
755 760 765

Ile Asn Lys Ser Ala Leu Glu Thr Lys Thr Thr Thr Lys Leu Ser Met  
770 775 780

Leu Glu Leu His Gly Asn Pro Phe Glu Cys Thr Cys Asp Ile Gly Asp  
785 790 795 800

Phe Arg Arg Trp Met Asp Glu His Leu Asn Val Lys Ile Pro Arg Leu  
805 810 815

Val Asp Val Ile Cys Ala Ser Pro Gly Asp Gln Arg Gly Lys Ser Ile  
820 825 830

Val Ser Leu Glu Leu Thr Thr Cys Val Ser Asp Val Thr Ala Val Ile  
835 840 845

Leu Phe Phe Phe Thr Phe Phe Ile Thr Thr Met Val Met Leu Ala Ala  
 850 855 860  
 Leu Ala His His Leu Phe Tyr Trp Asp Val Trp Phe Ile Tyr Asn Val  
 865 870 875 880  
 Cys Leu Ala Lys Ile Lys Gly Tyr Arg Ser Leu Ser Thr Ser Gln Thr  
 885 890 895  
 Phe Tyr Asp Ala Tyr Ile Ser Tyr Asp Thr Lys Asp Ala Ser Val Thr  
 900 905 910  
 Asp Trp Val Ile Asn Glu Leu Arg Tyr His Leu Glu Glu Ser Arg Asp  
 915 920 925  
 Lys Asn Val Leu Leu Cys Leu Glu Glu Arg Asp Trp Asp Pro Gly Leu  
 930 935 940  
 Ala Ile Ile Asp Asn Leu Met Gln Ser Ile Asn Gln Ser Lys Lys Thr  
 945 950 955 960  
 Val Phe Val Leu Thr Lys Lys Tyr Ala Lys Ser Trp Asn Phe Lys Thr  
 965 970 975  
 Ala Phe Tyr Leu Ala Leu Gln Arg Leu Met Asp Glu Asn Met Asp Val  
 980 985 990  
 Ile Ile Phe Ile Leu Leu Glu Pro Val Leu Gln His Ser Gln Tyr Leu  
 995 1000 1005  
 Arg Leu Arg Gln Arg Ile Cys Lys Ser Ser Ile Leu Gln Trp Pro  
 1010 1015 1020  
 Asp Asn Pro Lys Ala Glu Gly Leu Phe Trp Gln Thr Leu Arg Asn  
 1025 1030 1035  
 Val Val Leu Thr Glu Asn Asp Ser Arg Tyr Asn Asn Met Tyr Val  
 1040 1045 1050  
 Asp Ser Ile Lys Gln Tyr  
 1055

<210> 31  
 <211> 3367

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 31

```

ctcctgcata gagggtagca ttctgcgctg ctgcaagtta cggaatgaaa aattagaaca      60
acagaaacgt gggtctcttg acacttcagt gttagggaac atcagcaaga cccatcccag      120
gagaccttga aggaagcctt tgaaagggag aatgaaggag tcatctttgc aaaatagctc      180
ctgcagcctg ggaaaggaga ctaaaaagga aaacatgttc cttcagtcgt caatgctgac      240
ctgcattttc ctgctaatat ctggttcctg tgagttatgc gccgaagaaa atttttctag      300
aagctatcct tgtgatgaga aaaagcaaaa tgactcagtt attgcagagt gcagcaatcg      360
tcgactacag gaagttcccc aaacgggtggg caaatatgtg acagaactag acctgtctga      420
taatttcctc acacacataa cgaatgaatc atttcaaggg ctgcaaaatc tcaactaaaat      480
aaatctaaac cacaacccca atgtacagca ccagaacgga aatcccggta tacaatcaaa      540
tggtctgaat atcacagacg gggcattcct caacctaaaa aacctaaagg agttactgct      600
tgaagacaac cagttacccc aaataccctc tggtttgcca gagtctttga cagaacttag      660
tctaattcaa aacaatatat acaacataac taaagagggc atttcaagac ttataaactt      720
gaaaaatctc tatttggcct ggaactgcta ttttaacaaa gtttgcgaga aaactaacat      780
agaagatgga gtatttgaaa cgctgacaaa tttggagttg ctatcactat ctttcaattc      840
tctttcacac gtgtcaccca aactgccaa gctccctacgc aaactttttc tgagcaacac      900
ccagatcaaa tacattagtg aagaagattt caagggattg ataaatttaa cattactaga      960
tttaagcggg aactgtccga ggtgcttcaa tgccccattt ccatgcgtgc cttgtgatgg     1020
tggtgcttca attaatatag atcgttttgc ttttcaaaac ttgaccaaac ttcgatacct     1080
aaacctctct agcacttccc tcaggaagat taatgctgcc tggtttaaaa atatgcctca     1140
tctgaagggt ctggatcttg aattcaacta tttagtggga gaaatagcct ctggggcatt     1200
tttaacgatg ctgccccgct tagaaatact tgacttgtct tttactata taaaggggag     1260
ttatccacag catattaata tttccagaaa cttctctaaa cttttgtctc tacgggcatt     1320
gcatttaaga gggtatgtgt tccaggaact cagagaagat gatttccagc ccctgatgca     1380
gcttccaaac ttatcgacta tcaacttggg tattaatttt attaaagcaa tcgatttcaa     1440
acttttccaa aatttctcca atctggaaat tatttacttg tcagaaaaca gaatatcacc     1500
gttggtaaaa gataccggc agagttagtc aaatagttcc tcttttcaac gtcatatccg     1560
gaaacgacgc tcaacagatt ttgagtttga cccacattcg aacttttate atttcacccg     1620
tcctttaata aagccacaat gtgctgctta tggaaaagcc ttagatttaa gcctcaacag     1680

```

tatttttcttc attgggccaa accaatttga aaatcttcct gacattgcct gtttaaactct	1740
gtctgcacaa agcaatgctc aagtgttaag tggaactgaa ttttcagcca ttctcatgt	1800
caaataatttg gatttgacaa acaatagact agactttgat aatgctagtg ctcttactga	1860
attgtccgac ttggaagttc tagatctcag ctataattca cactatttca gaatagcagg	1920
cgtaacacat catctagaat ttattcaaaa tttcacaaat ctaaaagttt taaacttgag	1980
ccacaacaac atttatactt taacagataa gtataacctg gaaagcaagt ccctggtaga	2040
attagttttc agtggcaatc gccttgacat tttgtggaat gatgatgaca acaggtatat	2100
ctccattttc aaaggctcct agaactctgac acgtctggat ttatccctta ataggctgaa	2160
gcacatccca aatgaagcat tccttaattt gccagcgagt ctactgaac tacatataaa	2220
tgataatatg ttaaagtttt ttaactggac attactccag cagtttcttc gtctcgagtt	2280
gcttgactta cgtggaaaca aactactctt ttttaactgat agcctatctg actttacatc	2340
ttcccttcgg aactgctgc tgagtcataa caggatttcc cacctaccct ctggctttct	2400
ttctgaagtc agtagtctga agcacctcga ttttaagttcc aatctgctaa aaacaatcaa	2460
caaatccgca cttgaaacta agaccaccac caaattatct atgttggaac tacacggaaa	2520
cccctttgaa tgcacctgtg acattggaga tttccgaaga tggatggatg aacatctgaa	2580
tgtcaaaatt ccagactgg tagatgtcat ttgtgccagt cctggggatc aaagagggaa	2640
gagtattgtg agtctggagc taacaacttg tgtttcagat gtcactgcag tgatattatt	2700
tttcttcacg ttctttatca ccaccatggt tatgttggct gccctggctc accatttggt	2760
ttactgggat gtttggttta tatataatgt gtgttttagct aagataaaag gctacaggtc	2820
tctttccaca tcccaaactt tctatgatgc ttacatttct tatgacacca aagatgcctc	2880
tgttactgac tgggtgataa atgagctgcg ctaccacctt gaagagagcc gagacaaaaa	2940
cgttctcctt tgtctagagg agagggattg ggaccggga ttggccatca tcgacaacct	3000
catgcagagc atcaaccaaa gcaagaaaac agtatattgtt ttaacaaaaa aatatgcaaa	3060
aagctggaac tttaaaacag ctttttactt ggctttgcag aggctaattg atgagaacat	3120
ggatgtgatt atatttatcc tgctggagcc agtggttacag cattctcagt atttgaggct	3180
acggcagcgg atctgtaaga gctccatcct ccagtggcct gacaaccga aggcagaagg	3240
cttggttttg caaactctga gaaatgtggt cttgactgaa aatgattcac ggtataacaa	3300
tatgtatgtc gattccatta agcaatacta actgacgtta agtcatgatt tcgcgccata	3360
ataaaga	3367

<210> 32  
 <211> 1032  
 <212> PRT  
 <213> Mus musculus

<400> 32

Met Glu Asn Met Pro Pro Gln Ser Trp Ile Leu Thr Cys Phe Cys Leu  
 1 5 10 15

Leu Ser Ser Gly Thr Ser Ala Ile Phe His Lys Ala Asn Tyr Ser Arg  
 20 25 30

Ser Tyr Pro Cys Asp Glu Ile Arg His Asn Ser Leu Val Ile Ala Glu  
 35 40 45

Cys Asn His Arg Gln Leu His Glu Val Pro Gln Thr Ile Gly Lys Tyr  
 50 55 60

Val Thr Asn Ile Asp Leu Ser Asp Asn Ala Ile Thr His Ile Thr Lys  
 65 70 75 80

Glu Ser Phe Gln Lys Leu Gln Asn Leu Thr Lys Ile Asp Leu Asn His  
 85 90 95

Asn Ala Lys Gln Gln His Pro Asn Glu Asn Lys Asn Gly Met Asn Ile  
 100 105 110

Thr Glu Gly Ala Leu Leu Ser Leu Arg Asn Leu Thr Val Leu Leu Leu  
 115 120 125

Glu Asp Asn Gln Leu Tyr Thr Ile Pro Ala Gly Leu Pro Glu Ser Leu  
 130 135 140

Lys Glu Leu Ser Leu Ile Gln Asn Asn Ile Phe Gln Val Thr Lys Asn  
 145 150 155 160

Asn Thr Phe Gly Leu Arg Asn Leu Glu Arg Leu Tyr Leu Gly Trp Asn  
 165 170 175

Cys Tyr Phe Lys Cys Asn Gln Thr Phe Lys Val Glu Asp Gly Ala Phe  
 180 185 190

Lys Asn Leu Ile His Leu Lys Val Leu Ser Leu Ser Phe Asn Asn Leu  
 195 200 205

Phe Tyr Val Pro Pro Lys Leu Pro Ser Ser Leu Arg Lys Leu Phe Leu  
 210 215 220

Ser Asn Ala Lys Ile Met Asn Ile Thr Gln Glu Asp Phe Lys Gly Leu  
 225 230 235 240

Glu Asn Leu Thr Leu Leu Asp Leu Ser Gly Asn Cys Pro Arg Cys Tyr  
 245 250 255

Asn Ala Pro Phe Pro Cys Thr Pro Cys Lys Glu Asn Ser Ser Ile His  
 260 265 270

275

280

285

Leu Ser Ser Thr Ser Leu Arg Thr Ile Pro Ser Thr Trp Phe Glu Asn  
 290 295 300

Leu Ser Asn Leu Lys Glu Leu His Leu Glu Phe Asn Tyr Leu Val Gln  
 305 310 315 320

Glu Ile Ala Ser Gly Ala Phe Leu Thr Lys Leu Pro Ser Leu Gln Ile  
 325 330 335

Leu Asp Leu Ser Phe Asn Phe Gln Tyr Lys Glu Tyr Leu Gln Phe Ile  
 340 345 350

Asn Ile Ser Ser Asn Phe Ser Lys Leu Arg Ser Leu Lys Lys Leu His  
 355 360 365

Leu Arg Gly Tyr Val Phe Arg Glu Leu Lys Lys Lys His Phe Glu His  
 370 375 380

Leu Gln Ser Leu Pro Asn Leu Ala Thr Ile Asn Leu Gly Ile Asn Phe  
 385 390 395 400

Ile Glu Lys Ile Asp Phe Lys Ala Phe Gln Asn Phe Ser Lys Leu Asp  
 405 410 415

Val Ile Tyr Leu Ser Gly Asn Arg Ile Ala Ser Val Leu Asp Gly Thr  
 420 425 430

Asp Tyr Ser Ser Trp Arg Asn Arg Leu Arg Lys Pro Leu Ser Thr Asp  
 435 440 445

Asp Asp Glu Phe Asp Pro His Val Asn Phe Tyr His Ser Thr Lys Pro  
 450 455 460

Leu Ile Lys Pro Gln Cys Thr Ala Tyr Gly Lys Ala Leu Asp Leu Ser  
 465 470 475 480

Leu Asn Asn Ile Phe Ile Ile Gly Lys Ser Gln Phe Glu Gly Phe Gln  
 485 490 495

Asp Ile Ala Cys Leu Asn Leu Ser Phe Asn Ala Asn Thr Gln Val Phe  
 500 505 510

Asn Gly Thr Glu Phe Ser Ser Met Pro His Ile Lys Tyr Leu Asp Leu  
 515 520 525

Thr Asn Asn Arg Leu Asp Phe Asp Asp Asn Asn Ala Phe Ser Asp Leu  
 530 535 540

His Asp Leu Glu Val Leu Asp Leu Ser His Asn Ala His Tyr Phe Ser  
 545 550 555 560

Ile Ala Gly Val Thr His Arg Leu Gly Phe Ile Gln Asn Leu Ile Asn  
 565 570 575

Leu Arg Val Leu Asn Leu Ser His Asn Gly Ile Tyr Thr Leu Thr Glu  
 580 585 590

Glu Ser Glu Leu Lys Ser Ile Ser Leu Lys Glu Leu Val Phe Ser Gly  
 595 600 605

Asn Arg Leu Asp His Leu Trp Asn Ala Asn Asp Gly Lys Tyr Trp Ser  
 610 615 620

Ile Phe Lys Ser Leu Gln Asn Leu Ile Arg Leu Asp Leu Ser Tyr Asn  
 625 630 635 640

Asn Leu Gln Gln Ile Pro Asn Gly Ala Phe Leu Asn Leu Pro Gln Ser  
 645 650 655

Leu Gln Glu Leu Leu Ile Ser Gly Asn Lys Leu Arg Phe Phe Asn Trp  
 660 665 670

Thr Leu Leu Gln Tyr Phe Pro His Leu His Leu Leu Asp Leu Ser Arg  
 675 680 685

Asn Glu Leu Tyr Phe Leu Pro Asn Cys Leu Ser Lys Phe Ala His Ser  
690 695 700

Leu Glu Thr Leu Leu Leu Ser His Asn His Phe Ser His Leu Pro Ser  
705 710 715 720

Gly Phe Leu Ser Glu Ala Arg Asn Leu Val His Leu Asp Leu Ser Phe  
725 730 735

Asn Thr Ile Lys Met Ile Asn Lys Ser Ser Leu Gln Thr Lys Met Lys  
740 745 750

Thr Asn Leu Ser Ile Leu Glu Leu His Gly Asn Tyr Phe Asp Cys Thr  
755 760 765

Cys Asp Ile Ser Asp Phe Arg Ser Trp Leu Asp Glu Asn Leu Asn Ile  
770 775 780

785 790 795 800

Lys Ser Lys Ser Ile Met Ser Leu Asp Leu Thr Thr Cys Val Ser Asp  
805 810 815

Thr Thr Ala Ala Val Leu Phe Phe Leu Thr Phe Leu Thr Thr Ser Met  
820 825 830

Val Met Leu Ala Ala Leu Val His His Leu Phe Tyr Trp Asp Val Trp  
835 840 845

Phe Ile Tyr His Met Cys Ser Ala Lys Leu Lys Gly Tyr Arg Thr Ser  
850 855 860

Ser Thr Ser Gln Thr Phe Tyr Asp Ala Tyr Ile Ser Tyr Asp Thr Lys  
865 870 875 880

Asp Ala Ser Val Thr Asp Trp Val Ile Asn Glu Leu Arg Tyr His Leu  
885 890 895

Glu Glu Ser Glu Asp Lys Ser Val Leu Leu Cys Leu Glu Glu Arg Asp  
900 905 910

Trp Asp Pro Gly Leu Pro Ile Ile Asp Asn Leu Met Gln Ser Ile Asn  
915 920 925

Gln Ser Lys Lys Thr Ile Phe Val Leu Thr Lys Lys Tyr Ala Lys Ser  
 930 935 940

Trp Asn Phe Lys Thr Ala Phe Tyr Leu Ala Leu Gln Arg Leu Met Asp  
 945 950 955 960

Glu Asn Met Asp Val Ile Ile Phe Ile Leu Leu Glu Pro Val Leu Gln  
 965 970 975

Tyr Ser Gln Tyr Leu Arg Leu Arg Gln Arg Ile Cys Lys Ser Ser Ile  
 980 985 990

Leu Gln Trp Pro Asn Asn Pro Lys Ala Glu Asn Leu Phe Trp Gln Ser  
 995 1000 1005

Leu Lys Asn Val Val Leu Thr Glu Asn Asp Ser Arg Tyr Asp Asp  
 1010 1015 1020

Leu Tyr Ile Asp Ser Ile Arg Gln Tyr  
 1025 1030

<210> 33

<211> 3220

<212> DNA

<213> Mus musculus

<400> 33

```

attcagagtt ggatgttaag agagaaacaa acgtttttacc ttcctttgtc tatagaacat      60
ggaaaacatg cccctcagt catggattct gacgtgcttt tgtctgctgt cctctggaac      120
cagtgccatc ttccataaag cgaactattc cagaagctat ccttgtgacg agataaggca      180
caactccctt gtgattgcag aatgcaacca tcgtcaactg catgaagtgc cccaaactat      240
aggcaagtat gtgacaaaca tagacttgtc agacaatgcc attacacata taacgaaaga      300
gtcctttcaa aagctgcaaa acctcactaa aatcgatctg aaccacaatg ccaaacaaca      360
gcacccaaat gaaaataaaa atggtatgaa tattacagaa ggggcacttc tcagcctaag      420
aaatctaaca gttttactgc tggaagacaa ccagttatat actatacctg ctggggtgac      480
tgagtctttg aaagaactta gcctaattca aaacaatata tttcaggtaa ctaaaaacaa      540
cacttttggg cttaggaact tggaaagact ctatttgggc tggaactgct attttaaatg      600
taatcaaacc ttttaaggtag aagatggggc atttaaaaat cttatacact tgaagggtact      660
ctcattatct ttcaataacc ttttctatgt gccccccaaa ctaccaagtt ctctaaggaa      720

```

actttttctg agtaatgcc	aatcatgaa catcactcag	gaagacttca aaggactgga	780
aaattttaaca ttactagatc	tgagtggaaa ctgtccaagg	tgttacaatg ctccatttcc	840
ttgcacacct tgcaaggaaa	actcatccat ccacatacat	cctctggctt ttcaaagtct	900
cacccaactt ctctatctaa	acctttccag cacttcctc	aggacgattc cttctacctg	960
gtttgaaaat ctgtcaaate	tgaaggaact ccactctgaa	ttcaactatt tagttcaaga	1020
aattgcctcg ggggcatttt	taacaaaact acccagttta	caaatecttg atttgtcctt	1080
caactttcaa tataaggaat	atttacaatt tattaatatt	tcctcaaatt tctctaagct	1140
tcgttctctc aagaagttgc	acttaagagg ctatgtgttc	cgagaactta aaaagaagca	1200
tttcgagcat ctccagagtc	ttccaaactt ggcaaccatc	aacttgggca ttaactttat	1260
tgagaaaatt gatttcaaag	ctttccagaa tttttccaaa	ctcgacgtta tctattttatc	1320
aggaaatcgc atagcatctg	tattagatgg tacagattat	tcctcttggc gaaatcgtct	1380
tcggaaacct ctctcaacag	acgatgatga gtttgatcca	cacgtgaatt tttaccatag	1440
caccaaacct ttaataaagc	cacagtgtac tgcttatggc	aaggccttgg attttaagttt	1500
gaacaatatt ttcattattg	ggaaaagcca atttgaaggt	tttcaggata tcgcctgctt	1560
aaatctgtcc ttcaatgcc	atactcaagt gtttaatggc	acagaattct cctccatgcc	1620
ccacattaaa tatttggtg	taaccaacaa cagactagac	tttgatgata acaatgcttt	1680
cagtgatctt cagcatctag	aagtgtctga cctgagccac	aatgcacact atttcagtat	1740
agcaggggta acgcaccgtc	taggatttat ccagaactta	ataaacctca ggggtgttaa	1800
cctgagccac aatggcattt	acaccctcac agaggaaagt	gagctgaaaa gcatctcact	1860
gaaagaattg gttttcagt	gaaatcgtct tgaccatttg	tggaatgcaa atgatggcaa	1920
atactggtcc atttttaaaa	gtctccagaa tttgatacgc	ctggacttat catacaataa	1980
ccttcaacaa atcccaaag	gagcattcct caatttgcct	cagagcctcc aagagttact	2040
tatcagtggt aacaaattac	gtttctttaa ttggacatta	ctccagtatt ttcctcacct	2100
tgacatttcc ctggagacac	tgctactgag ccataatcat	ttctctcacc taccctctgg	2220
cttcctctcc gaagccagga	atctggtgca cctggatcta	agtttcaaca caataaagat	2280
gatcaataaa tcctccctgc	aaaccaagat gaaaacgaac	ttgtctattc tggagctaca	2340
tggaacttat tttgactgca	cgtgtgacat aagtgtttt	cgaagctggc tagatgaaaa	2400
tctgaatatc acaattccta	aattggtaaa tggttatatg	tccaatcctg gggatcaaaa	2460
atcaaagagt atcatgagcc	tagatctcac gacttgtgta	tcggatacca ctgcagctgt	2520

```

cctgtttttc ctcacattcc ttaccacctc catgggttatg ttggctgctc tggttcacca 2580
cctgtttttac tgggatgttt ggtttatcta tcacatgtgc tctgctaagt taaaaggcta 2640
caggacttca tccacatccc aaactttcta tgatgcttat atttcttatg acaccaaaga 2700
tgcattctgtt actgactggg taatcaatga actgctgtac caccttgaag agagtgaaga 2760
caaaagtgtc ctcctttgtt tagaggagag ggattgggat ccaggattac ccatcattga 2820
taacctcatg cagagcataa accagagcaa gaaaacaatc tttgttttaa ccaagaaata 2880
tgccaagagc tggaacttta aaacagcttt ctacttggcc ttgcagaggc taatggatga 2940
gaacatggat gtgattatth tcatcctcct ggaaccagtg ttacagtact cacagtacct 3000
gaggcttcgg cagaggatct gtaagagctc catcctccag tggcccaaca atcccaaagc 3060
agaaaacttg ttttggcaaa gtctgaaaaa tgtggtcttg actgaaaatg attcacggta 3120
tgacgatttg tacattgatt ccattaggca atactagtga tgggaagtca cgactctgcc 3180
atcataaaaa cacacagctt ctccttacia tgaaccgaat 3220

```

```

<210> 34
<211> 1032
<212> PRT
<213> Homo sapiens

```

```

<400> 34

```

```

Met Gly Phe Cys Arg Ser Ala Leu His Pro Leu Ser Leu Leu Val Gln
1           5           10           15

```

```

Ala Ile Met Leu Ala Met Thr Leu Ala Leu Gly Thr Leu Pro Ala Phe
20           25           30

```

```

Leu Pro Cys Glu Leu Gln Pro His Gly Leu Val Asn Cys Asn Trp Leu
35           40           45

```

```

Phe Leu Lys Ser Val Pro His Phe Ser Met Ala Ala Pro Arg Gly Asn
50           55           60

```

```

Val Thr Ser Leu Ser Leu Ser Ser Asn Arg Ile His His Leu His Asp
65           70           75           80

```

```

Ser Asp Phe Ala His Leu Pro Ser Leu Arg His Leu Asn Leu Lys Trp
85           90           95

```

```

Asn Cys Pro Pro Val Gly Leu Ser Pro Met His Phe Pro Cys His Met
100          105          110

```

Thr Ile Glu Pro Ser Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu  
 115 120 125

Asn Leu Ser Tyr Asn Asn Ile Met Thr Val Pro Ala Leu Pro Lys Ser  
 130 135 140

Leu Ile Ser Leu Ser Leu Ser His Thr Asn Ile Leu Met Leu Asp Ser  
 145 150 155 160

Ala Ser Leu Ala Gly Leu His Ala Leu Arg Phe Leu Phe Met Asp Gly  
 165 170 175

Asn Cys Tyr Tyr Lys Asn Pro Cys Arg Gln Ala Leu Glu Val Ala Pro

Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr  
 195 200 205

Asn Asn Leu Thr Val Val Pro Arg Asn Leu Pro Ser Ser Leu Glu Tyr  
 210 215 220

Leu Leu Leu Ser Tyr Asn Arg Ile Val Lys Leu Ala Pro Glu Asp Leu  
 225 230 235 240

Ala Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg  
 245 250 255

Arg Cys Asp His Ala Pro Asn Pro Cys Met Glu Cys Pro Arg His Phe  
 260 265 270

Pro Gln Leu His Pro Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly  
 275 280 285

Leu Val Leu Lys Asp Ser Ser Leu Ser Trp Leu Asn Ala Ser Trp Phe  
 290 295 300

Arg Gly Leu Gly Asn Leu Arg Val Leu Asp Leu Ser Glu Asn Phe Leu  
 305 310 315 320

Tyr Lys Cys Ile Thr Lys Thr Lys Ala Phe Gln Gly Leu Thr Gln Leu  
 325 330 335

Arg Lys Leu Asn Leu Ser Phe Asn Tyr Gln Lys Arg Val Ser Phe Ala  
 340 345 350

His Leu Ser Leu Ala Pro Ser Phe Gly Ser Leu Val Ala Leu Lys Glu  
 355 360 365

Leu Asp Met His Gly Ile Phe Phe Arg Ser Leu Asp Glu Thr Thr Leu  
 370 375 380

Arg Pro Leu Ala Arg Leu Pro Met Leu Gln Thr Leu Arg Leu Gln Met  
 385 390 395 400

Asn Phe Ile Asn Gln Ala Gln Leu Gly Ile Phe Arg Ala Phe Pro Gly  
 405 410 415

Leu Arg Tyr Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ser Glu  
 420 425 430

Leu Thr Ala Thr Met Gly Glu Ala Asp Gly Gly Glu Lys Val Trp Leu  
 435 440 445

Gln Pro Gly Asp Leu Ala Pro Ala Pro Val Asp Thr Pro Ser Ser Glu  
 450 455 460

Asp Phe Arg Pro Asn Cys Ser Thr Leu Asn Phe Thr Leu Asp Leu Ser  
 465 470 475 480

Arg Asn Asn Leu Val Thr Val Gln Pro Glu Met Phe Ala Gln Leu Ser  
 485 490 495

His Leu Gln Cys Leu Arg Leu Ser His Asn Cys Ile Ser Gln Ala Val  
 500 505 510

Asn Gly Ser Gln Phe Leu Pro Leu Thr Gly Leu Gln Val Leu Asp Leu  
 515 520 525

Ser His Asn Lys Leu Asp Leu Tyr His Glu His Ser Phe Thr Glu Leu  
 530 535 540

Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Gly  
 545 550 555 560

Met Gln Gly Val Gly His Asn Phe Ser Phe Val Ala His Leu Arg Thr  
 565 570 575

Leu Arg His Leu Ser Leu Ala His Asn Asn Ile His Ser Gln Val Ser  
 580 585 590

Gln Gln Leu Cys Ser Thr Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn  
 595 600 605

Ala Leu Gly His Met Trp Ala Glu Gly Asp Leu Tyr Leu His Phe Phe  
 610 615 620

Gln Gly Leu Ser Gly Leu Ile Trp Leu Asp Leu Ser Gln Asn Arg Leu  
 625 630 635 640

His Thr Leu Leu Pro Gln Thr Leu Arg Asn Leu Pro Lys Ser Leu Gln  
 645 650 655

Val Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Lys Trp Trp Ser  
 660 665 670

Leu His Phe Leu Pro Lys Leu Glu Val Leu Asp Leu Ala Gly Asn Gln  
 675 680 685

Leu Lys Ala Leu Thr Asn Gly Ser Leu Pro Ala Gly Thr Arg Leu Arg

Arg Leu Asp Val Ser Cys Asn Ser Ile Ser Phe Val Ala Pro Gly Phe  
 705 710 715 720

Phe Ser Lys Ala Lys Glu Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala  
 725 730 735

Leu Lys Thr Val Asp His Ser Trp Phe Gly Pro Leu Ala Ser Ala Leu  
 740 745 750

Gln Ile Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala  
 755 760 765

Ala Phe Met Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu  
 770 775 780

Pro Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Leu Ser  
 785 790 795 800

Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Ala Leu Ser Trp  
 805 810 815

Asp Cys Phe Ala Leu Ser Leu Leu Ala Val Ala Leu Gly Leu Gly Val  
 820 825 830

Pro Met Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His  
835 840 845

Leu Cys Leu Ala Trp Leu Pro Trp Arg Gly Arg Gln Ser Gly Arg Asp  
850 855 860

Glu Asp Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Thr Gln  
865 870 875 880

Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Gly Gln Leu Glu  
885 890 895

Glu Cys Arg Gly Arg Trp Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp  
900 905 910

Trp Leu Pro Gly Lys Thr Leu Phe Glu Asn Leu Trp Ala Ser Val Tyr  
915 920 925

Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser  
930 935 940

Gly Leu Leu Arg Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu  
945 950 955 960

Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Ser Pro Asp Gly Arg  
965 970 975

Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val  
980 985 990

Leu Leu Trp Pro His Gln Pro Ser Gly Gln Arg Ser Phe Trp Ala Gln  
995 1000 1005

Leu Gly Met Ala Leu Thr Arg Asp Asn His His Phe Tyr Asn Arg  
1010 1015 1020

Asn Phe Cys Gln Gly Pro Thr Ala Glu  
1025 1030

<210> 35  
<211> 3257  
<212> DNA  
<213> Homo sapiens

<400> 35  
 ccgctgctgc ccctgtggga agggacctcg agtgtgaagc atccttcctt gtagctgctg 60  
 tccagtctgc ccgccagacc ctctggagaa gccccgtccc ccagcatgg gtttctgccc 120  
 cagcgccctg caccgcgtgt ctctcctggt gcaggccatc atgctggcca tgaccctggc 180  
 cctgggtacc ttgcctgcct tcctacctg tgagctccag cccacaggcc tggagaactg 240  
 caactggctg ttctgaagt ctgtgcccc cttctccatg gcagcaccgc gtggcaatgt 300  
 caccagcctt tccttgctct ccaaccgcat ccaccacctc catgattctg actttgcccc 360  
 cctgcccagc ctgcggcatc tcaacctcaa gtggaactgc ccgcgggtg gcctcagccc 420  
 catgcacttc ccctgccaca tgaccatcga gccagcacc ttcttggtg tgcccacct 480  
 ggaagagcta aacctgagct acaacaacat catgactgtg cctgcgctgc ccaaaccct 540  
 catatccctg tccctcagcc ataccaacat cctgatgcta gactctgcca gcctcgccgg 600  
 cctgcatgcc ctgcgcttcc tattcatgga cggcaactgt tattacaaga acccctgcag 660  
 gcaggcactg gaggtggccc cgggtgccct ccttggcctg ggcaacctca cccacctgtc 720  
 actcaagtac aacaacctca ctgtggtgcc ccgcaacctg cttccagcc tggagtatct 780  
 gctgtgtgcc tacaaccgca tcgtcaaact ggcgctgag gacctggcca atctgaccgc 840  
 cctgcgtgtg ctgatgtgg gcggaaattg ccgcgctgc gaccacgctc ccaaccctg 900  
 catggagtgc cctcgtaact tccccagct acatcccgat accttcagcc acctgagccg 960  
 tcttgaaggc ctggtgttga aggacagttc tctctcctgg ctgaatgcca gttggttccg 1020  
 tgggctggga aacctccgag tgctggacct gagtgagaac ttcctctaca aatgcatcac 1080  
 taaaaccaag gccttcagc gcctaacaca gctgcgcaag cttaacctgt cttcaatta 1140  
 caaaagagg gtgtcctttg cccacctgtc tctggccctt tccttcggga gcctggtcgc 1200  
 cctgaaggag ctggacatgc acggcatctt cttccgctca ctgatgaga ccacgctccg 1260  
 gccactggcc cgctgccc tgcctcagac tctgcgtctg cagatgaact tcatcaacca 1320  
 ggcccagctc ggcatcttca gggccttccc tggcctgcgc tacgtggacc tgcggacaa 1380  
 ccgcatcagc ggagcttcgg agctgacagc caccatgggg gaggcagatg gaggggagaa 1440  
 ggtctggctg cagcctgggg accttgctcc ggccccagtg gacactcca gctctgaaga 1500  
 cttcaggccc aactgcagca ccctcaactt caccttgat ctgtcacgga acaacctggt 1560  
 gaccgtgcag ccggagatgt ttgccagct ctgcacctg cagtgcctgc gcctgagcca 1620  
 caactgcac tcgcaggcag tcaatggctc ccagttcctg ccgctgaccg gtctgcaggt 1680  
 gctagacctg tcccacaata agctggacct ctaccacgag cactcattca cggagctacc 1740  
 acgactggag gccctggacc tcagctacaa cagccagccc tttggcatgc agggcgtggg 1800

```

ccacaacttc agcttcgtgg ctcacctgcg caccctgcg caccctagcc tggcccacaa 1860
caacatccac agccaagtgt cccagcagct ctgcagtacg tcgctgcggg ccttggactt 1920
cagcggcaat gcactgggac atatgtgggc cgagggagac ctctatctgc acttcttcca 1980
aggcctgagc ggtttgatct ggctggactt gtcccagaac cgctgcaca cctcctgcc 2040
ccaaaccttg cgcaacctcc ccaagagcct acaggtgctg cgtctccgtg acaattacct 2100
ggccttcttt aagtgggtga gcctccactt cctgccc aaa ctggaagtcc tcgacctggc 2160
aggaaaccag ctgaaggccc tgaccaatgg cagcctgcct gctggcaccg ggctccggag 2220
gctggatgtc agctgcaaca gcatcagctt cgtggccccc ggcttctttt ccaaggccaa 2280
ggagctgca gagctcaacc ttagcgccaa cgccctcaag acagtggacc actcctggtt 2340
tgggcccctg gcgagtggc tgcaataact agatgtaagc gccaaccctc tgactgcg 2400
ctgtggggcg gcctttatgg acttctgct ggaggtgcag gctgccgtgc ccggtctgcc 2460
cagccgggtg aagtgtggca gtccgggcca gctccagggc ctcagcatct ttgcacagga 2520
cctgcgcctc tgctggatg aggcctctc ctgggactgt ttgcctctc cgctgctggc 2580
tgtggctctg ggctgggtg tgccatgct gcatcacctc tgtggctggg acctctggt 2640
ctgcttccac ctgtgcctgg cctggcttcc ctggcggggg cggaagtg ggcgagatga 2700
ggatgcctg cctacgatg ccttcgtggg cttcgacaaa acgcagagcg cagtggcaga 2760
ctgggtgtac aacgagcttc gggggcagct ggaggagtgc cgtgggcgct gggcactccg 2820
cctgtgcctg gaggaacgcg actggctgcc tggcaaaacc ctctttgaga acctgtgggc 2880
ctcggctctat ggcagccgca agacgctgtt tgtgctggcc cacacggacc gggtcagtgg 2940
tctcttgcg gccagcttcc tgctggccca gcagcgctg ctggaggacc gcaaggacgt 3000
cgtgggtgctg gtgactctga gcctgacgg ccgcccgtcc cgctacgtgc ggctgcgcca 3060
gcgcctctgc cgccagagtg tcctcctctg gcccaccag cccagtggc agcgcagctt 3120
ctgggcccag ctgggcatgg cctgaccag ggacaaccac cacttctata accggaactt 3180
ctgccaggga cccacggccg aatagccgtg agccggaatc ctgcacggtg ccacctccac 3240
actcacctca cctctgc 3257

```

```

<210> 36
<211> 1055
<212> PRT
<213> Homo sapiens

<400> 36

```

Met Pro Met Lys Trp Ser Gly Trp Arg Trp Ser Trp Gly Pro Ala Thr  
 1 5 10 15  
 His Thr Ala Leu Pro Pro Pro Gln Gly Phe Cys Arg Ser Ala Leu His  
 20 25 30  
 Pro Leu Ser Leu Leu Val Gln Ala Ile Met Leu Ala Met Thr Leu Ala  
 35 40 45  
 Leu Gly Thr Leu Pro Ala Phe Leu Pro Cys Glu Leu Gln Pro His Gly  
 50 55 60  
 Leu Val Asn Cys Asn Trp Leu Phe Leu Lys Ser Val Pro His Phe Ser  
 65 70 75 80  
 Met Ala Ala Pro Arg Gly Asn Val Thr Ser Leu Ser Leu Ser Ser Asn  
 85 90 95  
 Arg Ile His His Leu His Asp Ser Asp Phe Ala His Leu Pro Ser Leu  
 100 105 110  
 Arg His Leu Asn Leu Lys Trp Asn Cys Pro Pro Val Gly Leu Ser Pro  
 115 120 125  
 Met His Phe Pro Cys His Met Thr Ile Glu Pro Ser Thr Phe Leu Ala  
 130 135 140  
 Val Pro Thr Leu Glu Glu Leu Asn Leu Ser Tyr Asn Asn Ile Met Thr  
 145 150 155 160  
 Val Pro Ala Leu Pro Lys Ser Leu Ile Ser Leu Ser Leu Ser His Thr  
 165 170 175  
 Asn Ile Leu Met Leu Asp Ser Ala Ser Leu Ala Gly Leu His Ala Leu  
 180 185 190  
 Arg Phe Leu Phe Met Asp Gly Asn Cys Tyr Tyr Lys Asn Pro Cys Arg  
 195 200 205  
 Gln Ala Leu Glu Val Ala Pro Gly Ala Leu Leu Gly Leu Gly Asn Leu  
 210 215 220  
 Thr His Leu Ser Leu Lys Tyr Asn Asn Leu Thr Val Val Pro Arg Asn  
 225 230 235 240

Leu Pro Ser Ser Leu Glu Tyr Leu Leu Leu Ser Tyr Asn Arg Ile Val  
 245 250 255

Lys Leu Ala Pro Glu Asp Leu Ala Asn Leu Thr Ala Leu Arg Val Leu  
 260 265 270

Asp Val Gly Gly Asn Cys Arg Arg Cys Asp His Ala Pro Asn Pro Cys  
 275 280 285

Met Glu Cys Pro Arg His Phe Pro Gln Leu His Pro Asp Thr Phe Ser  
 290 295 300

His Leu Ser Arg Leu Glu Gly Leu Val Leu Lys Asp Ser Ser Leu Ser  
 305 310 315 320

Trp Leu Asn Ala Ser Trp Phe Arg Gly Leu Gly Asn Leu Arg Val Leu  
 325 330 335

Asp Leu Ser Glu Asn Phe Leu Tyr Lys Cys Ile Thr Lys Thr Lys Ala  
 340 345 350

Phe Gln Gly Leu Thr Gln Leu Arg Lys Leu Asn Leu Ser Phe Asn Tyr  
 355 360 365

Gln Lys Arg Val Ser Phe Ala His Leu Ser Leu Ala Pro Ser Phe Gly  
 370 375 380

Ser Leu Val Ala Leu Lys Glu Leu Asp Met His Gly Ile Phe Phe Arg  
 385 390 395 400

Ser Leu Asp Glu Thr Thr Leu Arg Pro Leu Ala Arg Leu Pro Met Leu  
 405 410 415

Gln Thr Leu Arg Leu Gln Met Asn Phe Ile Asn Gln Ala Gln Leu Gly  
 420 425 430

Ile Phe Arg Ala Phe Pro Gly Leu Arg Tyr Val Asp Leu Ser Asp Asn  
 435 440 445

450

455

460

Gly Gly Glu Lys Val Trp Leu Gln Pro Gly Asp Leu Ala Pro Ala Pro  
 465 470 475 480

Val Asp Thr Pro Ser Ser Glu Asp Phe Arg Pro Asn Cys Ser Thr Leu  
 485 490 495

Asn Phe Thr Leu Asp Leu Ser Arg Asn Asn Leu Val Thr Val Gln Pro  
 500 505 510

Glu Met Phe Ala Gln Leu Ser His Leu Gln Cys Leu Arg Leu Ser His  
 515 520 525

Asn Cys Ile Ser Gln Ala Val Asn Gly Ser Gln Phe Leu Pro Leu Thr  
 530 535 540

Gly Leu Gln Val Leu Asp Leu Ser His Asn Lys Leu Asp Leu Tyr His  
 545 550 555 560

Glu His Ser Phe Thr Glu Leu Pro Arg Leu Glu Ala Leu Asp Leu Ser  
 565 570 575

Tyr Asn Ser Gln Pro Phe Gly Met Gln Gly Val Gly His Asn Phe Ser  
 580 585 590

Phe Val Ala His Leu Arg Thr Leu Arg His Leu Ser Leu Ala His Asn  
 595 600 605

Asn Ile His Ser Gln Val Ser Gln Gln Leu Cys Ser Thr Ser Leu Arg  
 610 615 620

Ala Leu Asp Phe Ser Gly Asn Ala Leu Gly His Met Trp Ala Glu Gly  
 625 630 635 640

Asp Leu Tyr Leu His Phe Phe Gln Gly Leu Ser Gly Leu Ile Trp Leu  
 645 650 655

Asp Leu Ser Gln Asn Arg Leu His Thr Leu Leu Pro Gln Thr Leu Arg  
 660 665 670

Asn Leu Pro Lys Ser Leu Gln Val Leu Arg Leu Arg Asp Asn Tyr Leu  
 675 680 685

Ala Phe Phe Lys Trp Trp Ser Leu His Phe Leu Pro Lys Leu Glu Val  
 690 695 700

Leu Asp Leu Ala Gly Asn Gln Leu Lys Ala Leu Thr Asn Gly Ser Leu  
 705 710 715 720

Pro Ala Gly Thr Arg Leu Arg Arg Leu Asp Val Ser Cys Asn Ser Ile  
                               725                              730                              735

Ser Phe Val Ala Pro Gly Phe Phe Ser Lys Ala Lys Glu Leu Arg Glu  
                               740                              745                              750

Leu Asn Leu Ser Ala Asn Ala Leu Lys Thr Val Asp His Ser Trp Phe  
                               755                              760                              765

Gly Pro Leu Ala Ser Ala Leu Gln Ile Leu Asp Val Ser Ala Asn Pro  
                               770                              775                              780

Leu His Cys Ala Cys Gly Ala Ala Phe Met Asp Phe Leu Leu Glu Val  
                               785                              790                              795                              800

Gln Ala Ala Val Pro Gly Leu Pro Ser Arg Val Lys Cys Gly Ser Pro  
                               805                              810                              815

Gly Gln Leu Gln Gly Leu Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys  
                               820                              825                              830

Leu Asp Glu Ala Leu Ser Trp Asp Cys Phe Ala Leu Ser Leu Leu Ala  
                               835                              840                              845

Val Ala Leu Gly Leu Gly Val Pro Met Leu His His Leu Cys Gly Trp  
                               850                              855                              860

Asp Leu Trp Tyr Cys Phe His Leu Cys Leu Ala Trp Leu Pro Trp Arg  
                               865                              870                              875                              880

Gly Arg Gln Ser Gly Arg Asp Glu Asp Ala Leu Pro Tyr Asp Ala Phe  
                               885                              890                              895

Val Val Phe Asp Lys Thr Gln Ser Ala Val Ala Asp Trp Val Tyr Asn  
                               900                              905                              910

Glu Leu Arg Gly Gln Leu Glu Glu Cys Arg Gly Arg Trp Ala Leu Arg  
                               915                              920                              925

Leu Cys Leu Glu Glu Arg Asp Trp Leu Pro Gly Lys Thr Leu Phe Glu  
                               930                              935                              940

Asn Leu Trp Ala Ser Val Tyr Gly Ser Arg Lys Thr Leu Phe Val Leu

945                      950                      955                      960  
                          965                      970                      975  
 Ala Gln Gln Arg Leu Leu Glu Asp Arg Lys Asp Val Val Val Leu Val  
                          980                      985                      990  
 Ile Leu Ser Pro Asp Gly Arg Arg Ser Arg Tyr Val Arg Leu Arg Gln  
                          995                      1000                      1005  
 Arg Leu Cys Arg Gln Ser Val Leu Leu Trp Pro His Gln Pro Ser  
                          1010                      1015                      1020  
 Gly Gln Arg Ser Phe Trp Ala Gln Leu Gly Met Ala Leu Thr Arg  
                          1025                      1030                      1035  
 Asp Asn His His Phe Tyr Asn Arg Asn Phe Cys Gln Gly Pro Thr  
                          1040                      1045                      1050  
 Ala Glu  
                          1055

<210> 37  
 <211> 3165  
 <212> DNA  
 <213> Homo sapiens

<400> 37  
 atgcccatga agtggagtgg gtggaggtgg agctgggggc cggccactca cacagccctc 60  
 ccacccccac agggtttctg ccgcagcgcc ctgcaccgcg tgtctctcct ggtgcaggcc 120  
 atcatgctgg ccatgaccct ggccctgggt accttgccctg ccttcctacc ctgtgagctc 180  
 cagccccacg gcctggtgaa ctgcaactgg ctgttcctga agtctgtgcc ccacttctcc 240  
 atggcagcac cccgtggcaa tgtcaccagc ctttccttgt cctccaaccg catccaccac 300  
 ctccatgatt ctgactttgc ccacctgccc agcctgcggc atctcaacct caagtggaac 360  
 tgcccgcgcg ttggcctcag ccccatgcac ttcccctgcc acatgaccat cgagcccagc 420  
 accttcttgg ctgtgcccac cctggaagag ctaaacctga gctacaacaa catcatgact 480  
 gtgcctgcgc tgcccaaata cctcatatcc ctgtccctca gccataccaa catcctgatg 540  
 ctagactctg ccagcctcgc cggcctgcat gccctgcgct tcctattcat ggacggcaac 600  
 tgttattaca agaaccctg caggeaggca ctggaggtgg ccccggtgct cctccttggc 660  
 ctgggcaacc tcaccacact gtcactcaag tacaacaacc tcaactgtgt gccccgaac 720

ctgccttcca gacctggagta tctgctgttg tctacaacc gcacgtcaa actgggcct	780
gaggacctgg ccaatctgac cgccctgctg gtgctcgatg tggcggaatg ttgccgccgc	840
tgcgaccacg ctcccaaccc ctgcatggag tgccctcgtc acttccccca gctacatccc	900
gataccttca gccacctgag ccgtcttgaa ggcctgggtg tgaaggacag ttctctctcc	960
tggctgaatg ccagttgggt ccgtgggctg ggaaacctcc gagtgctgga cctgagtgag	1020
aacttcctct acaaatgcat cactaaaacc aaggccttcc agggcctaac acagctgcgc	1080
aagcttaacc tgtccttcaa ttacaaaag aggggtgtct ttgccacct gtctctggcc	1140
ccttccttcg ggagcctggg cgccctgaag gagctggaca tgcacggcat cttcttcgcg	1200
tcactcgatg agaccacgt ccggccactg gccgcctgc ccatgctcca gactctgctg	1260
ctgcagatga acttcatcaa ccaggcccag ctcggcattc tcagggcctt ccctggcctg	1320
cgctacgtgg acctgtcga caaccgcatc agcggagctt cggagctgac agccaccatg	1380
ggggaggcag atggagggga gaaggctctg ctgcagcctg gggaccttgc tccggcccca	1440
gtggacactc ccagctctga agacttcagg cccaactgca gcacctcaa cttcaccttg	1500
gatctgtcac ggaacaacct ggtgaccgtg cagccggaga tgtttgcca gctctgcac	1560
ctgcagtgcc tgcgcctgag ccacaactgc atctcgagg cagtcaatgg ctcccagttc	1620
ctgccgtga ccggtctgca ggtgctagac ctgtcccaca ataagctgga cctctaccac	1680
gagcactcat tcacggagct accacgactg gaggccctgg acctcagcta caacagccag	1740
ccctttggca tgcagggcgt gggccacaac ttcagcttcg tggctcacct gcgcacctg	1800
cgccacctca gcctggccca caacaacatc cacagccaag tgtcccagca gctctgcagt	1860
acgtcgtgc gggccctgga cttcagcggc aatgcactgg gccatatgtg ggccgaggga	1920
gacctctatc tgcacttctt ccaaggcctg agcggtttga tctggctgga cttgtcccag	1980
aaccgcctgc acacctcct gcccacaacc ctgcgcaacc tccccagag cctacaggtg	2040
ctgcgtctcc gtgacaatta cctggccttc tttaagtggg ggagcctcca cttctgccc	2100
aaactggaag tcctcgacct ggcaggaaac cagctgaagg ccctgaccaa tggcagcctg	2160
cctgtggca ccggtctccg gaggtggat gtcagctgca acagcatcag cttcgtggcc	2220
cccggcttct tttccaaggc caaggagctg cgagagctca accttagcgc caacgcctc	2280
aagacagtgg accactcctg gtttgggccc ctggcgagtg ccctgcaaat actagatgta	2340
agcgccaacc ctctgactg cgctgtggg gcggccttta tggacttcct gctggaggtg	2400
caggctgccg tgcccgtct gccagccgg gtgaagtgtg gcagtccggg ccagctccag	2460

```

ggcctcagca tctttgcaca ggacctgcgc ctctgacctg atgaggccct ctctggggac 2520
tgtttcgccc tctcgtgctt ggctgtggct ctgggacctg gtgtgcccac gctgcatcac 2580
ctctgtggct gggacctctg gtactgcttc cacctgtgcc tggcctggct tccctggcgg 2640
gggcggcaaa gtgggcgaga tgaggatgcc ctgccctacg atgccttcgt ggtcttcgac 2700
aaaacgcaga gcgcagtggc agactgggtg tacaacgagc ttcgggggca gctggaggag 2760
tgccgtgggc gctgggcact ccgcctgtgc ctggaggaac gcgactggct gcctggcaaa 2820
accctctttg agaacctgtg ggacctcggtc tatggcagcc gcaagacgct gtttgtgctg 2880
gcccacacgg accgggtcag tggctctctg cgcgccagct tctgtctggc ccagcagcgc 2940
ctgctggagg accgcaagga cgctgtgggt ctggtgatcc tgagccctga cggccgccgc 3000
tcccgcctatg tgcggctgcg ccagcgcctc tgccgccaga gtgtcctcct ctggccccac 3060
cagcccagtg gtcagcgcag cttctgggcc cagctgggca tggccctgac cagggacaac 3120
caccacttct ataaccggaa cttctgccag ggacccacgg ccgaa 3165

```

```

<210> 38
<211> 1032
<212> PRT
<213> Mus musculus

```

```

<400> 38

```

```

Met Val Leu Arg Arg Arg Thr Leu His Pro Leu Ser Leu Leu Val Gln
1           5           10           15

```

```

Ala Ala Val Leu Ala Glu Thr Leu Ala Leu Gly Thr Leu Pro Ala Phe
20           25           30

```

```

Leu Pro Cys Glu Leu Lys Pro His Gly Leu Val Asp Cys Asn Trp Leu
35           40           45

```

```

Phe Leu Lys Ser Val Pro Arg Phe Ser Ala Ala Ala Ser Cys Ser Asn
50           55           60

```

```

Ile Thr Arg Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asn
65           70           75           80

```

```

Ser Asp Phe Val His Leu Ser Asn Leu Arg Gln Leu Asn Leu Lys Trp
85           90           95

```

```

Asn Cys Pro Pro Thr Gly Leu Ser Pro Leu His Phe Ser Cys His Met
100          105          110

```

Thr Ile Glu Pro Arg Thr Phe Leu Ala Met Arg Thr Leu Glu Glu Leu  
 115 120 125

Asn Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Arg Leu Pro Ser Ser  
 130 135 140

Leu Val Asn Leu Ser Leu Ser His Thr Asn Ile Leu Val Leu Asp Ala  
 145 150 155 160

Asn Ser Leu Ala Gly Leu Tyr Ser Leu Arg Val Leu Phe Met Asp Gly  
 165 170 175

Asn Cys Tyr Tyr Lys Asn Pro Cys Thr Gly Ala Val Lys Val Thr Pro  
 180 185 190

Gly Ala Leu Leu Gly Leu Ser Asn Leu Thr His Leu Ser Leu Lys Tyr  
 195 200 205

Asn Asn Leu Thr Tyr Lys Val Pro Arg Gln Leu Pro Pro Ser Leu Glu Tyr  
 210 215 220

Leu Leu Val Ser Tyr Asn Leu Ile Val Lys Leu Gly Pro Glu Asp Leu  
 225 230 235 240

Ala Asn Leu Thr Ser Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg  
 245 250 255

Arg Cys Asp His Ala Pro Asn Pro Cys Ile Glu Cys Gly Gln Lys Ser  
 260 265 270

Leu His Leu His Pro Glu Thr Phe His His Leu Ser His Leu Glu Gly  
 275 280 285

Leu Val Leu Lys Asp Ser Ser Leu His Thr Leu Asn Ser Ser Trp Phe  
 290 295 300

Gln Gly Leu Val Asn Leu Ser Val Leu Asp Leu Ser Glu Asn Phe Leu  
 305 310 315 320

Tyr Glu Ser Ile Asn His Thr Asn Ala Phe Gln Asn Leu Thr Arg Leu  
 325 330 335

Arg Lys Leu Asn Leu Ser Phe Asn Tyr Arg Lys Lys Val Ser Phe Ala  
 340 345 350

Arg Leu His Leu Ala Ser Ser Phe Lys Asn Leu Val Ser Leu Gln Glu  
 355 360 365

Leu Asn Met Asn Gly Ile Phe Phe Arg Ser Leu Asn Lys Tyr Thr Leu  
 370 375 380

Arg Trp Leu Ala Asp Leu Pro Lys Leu His Thr Leu His Leu Gln Met  
 385 390 395 400

Asn Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Thr Phe Arg Ala  
 405 410 415

Leu Arg Phe Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Pro Ser Thr  
 420 425 430

Leu Ser Glu Ala Thr Pro Glu Glu Ala Asp Asp Ala Glu Gln Glu Glu  
 435 440 445

Leu Leu Ser Ala Asp Pro His Pro Ala Pro Leu Ser Thr Pro Ala Ser  
 450 455 460

Lys Asn Phe Met Asp Arg Cys Lys Asn Phe Lys Phe Thr Met Asp Leu  
 465 470 475 480

Ser Arg Asn Asn Leu Val Thr Ile Lys Pro Glu Met Phe Val Asn Leu  
 485 490 495

Ser Arg Leu Gln Cys Leu Ser Leu Ser His Asn Ser Ile Ala Gln Ala  
 500 505 510

Val Asn Gly Ser Gln Phe Leu Pro Leu Thr Asn Leu Gln Val Leu Asp  
 515 520 525

Leu Ser His Asn Lys Leu Asp Leu Tyr His Trp Lys Ser Phe Ser Glu  
 530 535 540

Leu Pro Gln Leu Gln Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe  
 545 550 555 560

Ser Met Lys Gly Ile Gly His Asn Phe Ser Phe Val Ala His Leu Ser  
 565 570 575

Met Leu His Ser Leu Ser Leu Ala His Asn Asp Ile His Thr Arg Val  
 580 585 590

Ser Ser His Leu Asn Ser Asn Ser Val Arg Phe Leu Asp Phe Ser Gly  
595 600 605

Asn Gly Met Gly Arg Met Trp Asp Glu Gly Gly Leu Tyr Leu His Phe  
610 615 620

Phe Gln Gly Leu Ser Gly Leu Leu Lys Leu Asp Leu Ser Gln Asn Asn  
625 630 635 640

Leu His Ile Leu Arg Pro Gln Asn Leu Asp Asn Leu Pro Lys Ser Leu  
645 650 655

Lys Leu Leu Ser Leu Arg Asp Asn Tyr Leu Ser Phe Phe Asn Trp Thr  
660 665 670

Ser Leu Ser Phe Leu Pro Asn Leu Glu Val Leu Asp Leu Ala Gly Asn  
675 680 685

Gln Leu Lys Ala Leu Thr Asn Gly Thr Leu Pro Asn Gly Thr Leu Leu  
690 695 700

Gln Lys Leu Asp Val Ser Ser Asn Ser Ile Val Ser Val Val Pro Ala  
705 710 715 720

Phe Phe Ala Leu Ala Val Glu Leu Lys Glu Val Asn Leu Ser His Asn  
725 730 735

Ile Leu Lys Thr Val Asp Arg Ser Trp Phe Gly Pro Ile Val Met Asn  
740 745 750

Leu Thr Val Leu Asp Val Arg Ser Asn Pro Leu His Cys Ala Cys Gly  
755 760 765

Ala Ala Phe Val Asp Leu Leu Leu Glu Val Gln Thr Lys Val Pro Gly  
770 775 780

Leu Ala Asn Gly Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg  
785 790 795 800

Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Val Leu Ser  
805 810 815

Trp Asp Cys Phe Gly Leu Ser Leu Leu Ala Val Ala Val Gly Met Val

820	825	830
Val Pro Ile Leu His His Leu Cys Gly Trp Asp Val Trp Tyr Cys Phe		
835	840	845
His Leu Cys Leu Ala Trp Leu Pro Leu Leu Ala Arg Ser Arg Arg Ser		
850	855	860
Ala Gln Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln		
865	870	875
Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Arg Leu Glu		
	885	890
Glu Arg Arg Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Asp Arg Asp		
	900	905
Trp Leu Pro Gly Gln Thr Leu Phe Glu Asn Leu Trp Ala Ser Ile Tyr		
	915	920
Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser		
	930	935
Gly Leu Leu Arg Thr Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu		
945	950	955
Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Arg Pro Asp Ala His		
	965	970
Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val		
	980	985
Leu Phe Trp Pro Gln Gln Pro Asn Gly Gln Gly Gly Phe Trp Ala Gln		
	995	1000
Leu Ser Thr Ala Leu Thr Arg Asp Asn Arg His Phe Tyr Asn Gln		
1010	1015	1020
Asn Phe Cys Arg Gly Pro Thr Ala Glu		
1025	1030	

<210> 39  
 <211> 3200  
 <212> DNA  
 <213> Mus musculus

<400> 39  
 tgtcagaggg agcctcgga gaatcctcca tctcccaaca tggttctccg tcgaaggact 60  
 ctgcaccctt tgtccctcct ggtacaggct gcagtgtctg ctgagactct ggccttgggt 120  
 accctgcctg ccttctacc ctgtgagctg aagcctcatg gcctggtgga ctgcaattgg 180  
 ctgttctga agtctgtacc ccgtttctct gcggcagcat cctgtctcaa catcaccctg 240  
 ctctccttga tctccaaccg tatccaccac ctgcacaact ccgacttcgt ccacctgtcc 300  
 aacctgcggc agctgaacct caagtgaac tgtccacca ctggccttag cccctgcac 360  
 ctgaacctga gctataatgg tatcaccact gtgccccgac tgcccagctc cctggtgaat 480  
 ctgagcctga gccacaccaa catcctggtt ctagatgcta acagcctcgc cggcctatac 540  
 agcctgcgcg ttctcttcat ggacgggaac tgctactaca agaaccctg cacaggagcg 600  
 gtgaaggtga cccagggcgc cctcctgggc ctgagcaatc tcacccatct gtctctgaag 660  
 tataacaacc tcacaaaggt gcccgcgcaa ctgcccccca gcctggagta cctcctggtg 720  
 tcctataacc tcattgtcaa gctggggcct gaagacctgg ccaatctgac ctcccttcga 780  
 gtacttgatg tgggtgggaa ttgcgctcgc tgcgaccatg cccccaatcc ctgtatagaa 840  
 tgtggccaaa agtcctcca cctgcaccct gagaccttcc atcacctgag ccactctggaa 900  
 ggcttggctg tgaaggacag ctctctccat aactgaact cttcctggtt ccaaggctctg 960  
 gtcaacctct cgggtctgga cctaagcgag aactttctct atgaaagcat caaccacacc 1020  
 aatgccttcc agaacctaac ccgctgcgc aagctcaacc tgccttcaa ttaccgcaag 1080  
 aaggatcct ttgcccgcct ccacctggca agttccttca agaacctggg gtcactgcag 1140  
 gagctgaaca tgaacggcat cttcttccgc tcgctcaaca agtacacgct cagatggctg 1200  
 gccgatctgc ccaaactcca cactctgcat cttcaaatga acttcatcaa ccaggcacag 1260  
 ctgagcatct ttggtacctt ccgagccctt cgctttgttg acttgtcaga caatcgcatc 1320  
 agtgggcctt caacgctgtc agaagccacc cctgaagagg cagatgatgc agagcaggag 1380  
 gagctgttgt ctgcggatcc tcaccagct ccaactgagca cccctgcttc taagaacttc 1440  
 atggacaggt gtaagaactt caagttcacc atggacctgt ctcggaacaa cctggtgact 1500  
 atcaagccag agatgtttgt caatctctca cgcctccagt gtcttagcct gagccacaac 1560  
 tcattgcac aggtgtcaa tggtctcag ttctgcccgc tgactaatct gcaggtgctg 1620  
 gacctgtccc ataacaact ggacttgtac cactggaaat cgttcagtga gctaccacag 1680  
 ttgcaggccc tggacctgag ctacaacagc cagcccttta gcatgaaggg tataggccac 1740  
 aatttcagtt ttgtggccca tctgtccatg ctacacagcc ttagcctggc acacaatgac 1800

```

attcataccc gtgtgtcctc acatctcaac agcaactcag tgaggtttct tgacttcagc 1860
ggcaacggta tgggccgcat gtgggatgag gggggccttt atctccattt cttccaaggc 1920
ctgagtggcc tgctgaagct ggacctgtct caaaataacc tgcataatct ccggccccag 1980
aaccttgaca acctcccca gagcctgaag ctgctgagcc tccgagacaa ctacctatct 2040
ttctttaact ggaccagtct gtccttctctg cccaacctgg aagtcctaga cctggcaggc 2100
aaccagctaa aggccctgac caatggcacc ctgcctaata gcaccctcct ccagaaaactg 2160
gatgtcagca gcaacagtat cgtctctgtg gtcccagcct tcttcgctct ggcggtcgag 2220
ctgaaagagg tcaacctcag ccacaacatt ctcaagacgg tggatcgctc ctggtttggg 2280
cccattgtga tgaacctgac agttctagac gtgagaagca acctctgca ctgtgcctgt 2340
ggggcagcct tcgtagactt actgttggag gtgcagacca aggtgcctgg cctggctaata 2400
gggtgtgaagt gtggcagccc cggccagctg cagggccgta gcatcttcgc acaggacctg 2460
cggctgtgcc tggatgaggt cctctcttgg gactgctttg gcctttcact cttggctgtg 2520
gccgtgggca tgggtgggcc tatactgcac catctctgcg gctgggacgt ctggtactgt 2580
tttcatctgt gcctggcatg gctacctttg ctggcccgcg gccgacgcag cgccaagct 2640
ctcccctatg atgccttcgt ggtgttcgat aaggcacaga gcgcagttgc ggactgggtg 2700
tataacgagc tgcgggtgcg gctggaggag cggcgcggtc gccgagccct acgcttgtgt 2760
ctggaggacc gagattggct gcctggccag acgctcttcg agaacctctg ggcttccatc 2820
tatgggagcc gcaagactct atttgtgctg gccacacgg accgcgtcag tggcctcctg 2880
cgcaccagct tcctgctggc tcagcagcgc ctgttggaag accgcaagga cgtggtgggtg 2940
ttggtgatcc tgcgtccgga tgcccaccgc tcccgctatg tgcgactgcg ccagcgtctc 3000
tgccgccaga gtgtgctctt ctggccccag cagcccaacg ggcagggggg cttctgggcc 3060
cagctgagta cagccctgac tagggacaac cgccattctt ataaccagaa cttctgccgg 3120
ggacctacag cagaatagct cagagcaaca gctggaaaca gctgcatctt catgcctggt 3180
tcccagttg ctctgcctgc 3200

```